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Blood group studies in five lines of White Leghorns selected for single quantitative traits

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BLOOD GROUP STUDIES IN FIVE LINES OF WHITE LEGHORNS SELECTED FOR SINGLE QUANTITATIVE TRAITS

by

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INTRODUCTION

The possibility that the genes controlling blood group antigens may be associated with economically important traits has created considerable interest among geneticists engaged in animal and poultry improvement. The inheritance of quantitative traits are assumed to be controlled by the effects of many genes (polygenes, Mather, 1943) which may have pleiotropic effects and act as modifiers or suppressors of other genes (Lerner, 1958). The phenotypic expression of these genes may be modified by environmental forces. The variable effects of a single gene for a quantitative trait due to the genetic and environmental background in which it occurs, may make its presence or absence virtually impossible to determine phenotypically. In contrast, genes controlling the blood group antigens are observed, essentially, as dominants and their presence can be rather precisely determined. Should the genetic material controlling the blood group antigens be associated with economically important traits, then such blood group antigens could be used as economic trait indicators in a selection program. Regardless of associations with other traits, blood typing may have considerable value in studying genetic phenomena such as mutation, migration, selection, random drift, inbreeding, and linkage.

Commercial and experimental poultry breeding necessitates the development of lines that have been highly selected and, in many cases, inbred to a considerable degree. The possibility of

pedigree errors and accidental contamination of breeding stocks is considerable. Blood typing may be of great value to the commercial breeder or researcher in the maintenance of purity of lines by the accurate detection and elimination of spurious individuals.

The usual practice in making blood typing reagents is to inject intravenously, the red cells of one individual into a host individual of the same inbred line and collect the resulting antiserum of the host for use as a reagent. This procedure is called iso-immunization. Such an antiserum may be locus specific and yet react with various antigens controlled by a number of different alleles at that locus. In an inbred line where only a few alleles per locus segregate, the antiserum may identify one specific allele. Reagents so developed are generally suitable for use only within certain inbred lines. This is not a serious handicap to commercial breeders interested in blood typing a few highly inbred lines. However, chickens with little or no inbreeding cannot be satisfactorily blood typed with reagents made for use in inbred lines.

The purpose of this study is to develop reagents useful in establishing blood antigen genotypes in non-inbred lines of chickens and to use these reagents to study the effects of selection for quantitative traits on blood antigen genotypes. The account of the reagent development to be reported here will essentially be presented in three parts: (1) the development of specific

reagents, (2) the demonstration of the heritable nature of the antigens reacting with these reagents, and (3) the application of these reagents to characterize a population. The blood group gene frequencies of five lines, each selected for a different quantitative trait, will be studied to determine whether selection influenced blood group gene frequencies and particularly whether such influences are related to changes in body size and egg size.

This study also provides some basic information regarding methodology in developing blood typing reagents for use in non-inbred lines, and will indicate appropriate methods of utilizing these reagents. This makes it possible to study gene action in a population of chickens.

REVIEW OF LITERATURE

As early as 1875 Landois reported that mixing blood of one species with serum of another often caused red cells to agglutinate or clump together. Noting that sera from one man could agglutinate the red cells of another, Landsteiner (1900, 1901) discovered the ABO blood group system in man. Subsequent investigations indicate the existence of at least eight additional blood group systems. These systems and the discovery date as given by Cushing and Campbell (1957) are Rh, 1940; MNSs, 1918; P, 1927; Lutheran, 1945; Kell, 1946; Lewis, 1946; Duffy, 1950; and Kidd, 1951. In addition, there are a number of "private" or unique antigens peculiar to individual families as well as "public" antigens common to almost all individuals.

Epstein and Ottenberg (1908) suggested that the blood type of an individual was genetically determined. Two years later, von Dungern and Hirszfeld (1910) confirmed this theory. The work of Todd and White (1910) with cattle red cell antigens also indicated these to be genetically controlled. These investigators found that injecting cattle with foreign red cells produced antisera that, after adding fresh guinea pig serum, lysed the cells of some individuals. Injecting different animals with cells from the same donor produced antisera that differed in specificity for red cells of various individuals. While studying the wide variation between individuals they noted the similarity between parent and offspring. Landsteiner and Miller

(1924) adsorbed rabbit anti-chicken serum with the red cells of each of ten chickens and tested the other nine with the resulting adsorbed sera. They found that the cells could be divided into five distinct reaction patterns.

Todd (1930) found that injecting chickens with the red cells of other chickens produced antisera that strongly agglutinated chicken red cells. He found large individual differences in red cell reactivity to antisera produced by chickens and in antibody response to red cell injections. Todd (1930) found that antisera adsorbed with red cells from one parent failed to agglutinate the cells of about 25 per cent of the offspring, but that adsorption with cells from both parents made the antisera inactive for all offspring. Todd (1935) later showed that from brother sister matings it was possible to obtain families almost identical in reactivity to unadsorbed antisera. He concluded that the red cells of chickens possess a number of antigens which are inherited as dominants. Thomsen (1934, 1936) and Boyd and Alley (1940) reported isolated cases of individuals having an antigen not possessed by either parent. However, R. W. Briles (cited by Irwin, 1952) after repeating Thomsen's work, concluded that the exceptions reported above were due to faulty serological technique.

Blood Group Studies in Chickens

Briles *et al.* (1950b) reported the existence of two autosomal loci determining red cell antigens in chickens. These

were designated the A and B loci. Nine multiple alleles were reported for the A locus and five for the B. They proposed that each allele determines a total antigenic complex made up of a number of components. They suggested that these components were probably indicative of serological similarity between gene products but did not necessarily represent distinct entities.

Briles et al. (1950a) reported a third autosomal locus later designated C. A year later, Briles (1951) reported a fourth autosomal locus, D. Briles (1958) designated a fifth autosomal locus, E, which appeared to be closely linked to A with about one per cent crossing over. Seven apparent crossovers among 673 offspring from test matings were found. However, whether the A and E loci are really closely linked systems or represent one single blood group complex still has not been completely resolved.

Scheinberg (1956) reported two cases of possible linkage in chicken blood group loci. He found recombination frequencies of 5.4 $\pm$ 4.2 per cent between M and Q and 2.6 $\pm$ 2.0 per cent between O and S. Tests made with A and B reagents (supplied by Briles) indicated that Q probably corresponded with B. The relation of M, O, and S to Briles' reagents was not determined.

Gilmour (1958, 1959a) identified seven independent blood group loci segregating in a White Leghorn line inbred by fourteen successive brother-sister matings. Four loci corresponded to Briles' A, B, C, and E systems. Three other loci, designated L,

N, and Vh, were reported for the first time. The Vh locus was determined by agglutination reaction of red cells by vaccinia virus. Non agglutinability appeared to be caused by a single recessive gene.

Gilmour (1958) stated that attempts to manufacture specific lytic reagents by adsorption of anti-chicken immune rabbit sera were unsuccessful. He concluded that this confirmed an earlier study (Gilmour, 1949) indicating that adsorbed rabbit sera is "incapable of identifying the finer antigenic differences" that can be identified with iso-immune chicken serum.

Fanguy (1958) and Fanguy et al. (1961) attempted to blood type a closed flock of non-inbred White Leghorns using A, B, C, D, and E reagents made in three inbred White Leghorn lines. From one generation tested, tentative blood types were assigned based on reaction patterns using those reagents that discriminated between individuals. After testing the second generation (F_1) with the most discriminating reagents, the following blood group spectrum was postulated: two A alleles, five B alleles, three C alleles and four D alleles. Immunizations were made within the F_1 generation birds on the basis of tentatively assigned alleles. These immunizations produced one A reagent, five B reagents, and one or two C reagents.

Matsumoto and Okada (1961) found ten different antigenic factors by iso-immunization in chickens, presumed to be controlled by genes belonging to three non-linked loci designated F, G,

and H. These loci were not compared with previously designated loci.

Association of Blood Groups and Physiological Traits

A number of investigations have been undertaken to study the association of blood groups and physiological traits. Shultz and Briles (1953) studied the A and B blood group loci in a flock of chickens with low inbreeding. They found that in three generations of selection for high egg production, the selected groups contained a larger percentage of A locus heterozygotes than the total population. In addition, among those birds heterozygous at the A locus one B reagent identified a subgroup with higher egg production.

Briles et al. (1953) found that in two of three inbred Leghorn lines, the B heterozygous cockerels were significantly heavier than the homozygous cockerels at nine weeks of age. Later, Briles (1957) reported similar results for ten week old inbred line crosses. From preliminary data in a closed population of meat-type chickens, Kimmel (1960) reported that genes of the B blood group locus have an important effect on eight week body weight.

Briles (1954) studied the effects of blood group heterozygosity on hatchability. In three inbred lines over three seasons, the hatchability of fertile eggs laid by B locus heterozygous dams mated to B locus heterozygous sires was significantly

superior to eggs laid by homozygous dams and fertilized by homozygous males. Matings producing 100 per cent homozygotes were significantly lower in hatchability than matings expected to produce 50 per cent homozygotes. He found that the expectancy of heterozygote embryos hatching was about 1.7 times that of homozygous embryos. Briles also found that average egg production within each heterozygous line and season was from nine to 30 per cent greater than for homozygous hens. Briles (1956) reported essentially the same results for hen-day egg production for three seasons. Briles and Kruger (1955) stated that hatchability and chick viability was better from matings expected to produce 50 per cent B blood group heterozygotes than from those expected to produce all homozygotes.

Briles and Allen (1961) studied the effects of the B blood group genotype on livability and egg production in seven commercial inbred lines. They concluded that livability from fertilization to 150 days of age was associated with the B blood group genotype in two lines and that adult livability was associated with the B blood group genotype in two lines. Variation in rate of egg production was affected by the B blood group genotype in three lines.

Schierman and Nordskog (1961) reported finding a major histocompatibility locus in the chicken. Working with the A, B, C, D, and L blood group systems in an inbred line, they found that chicks would retain skin grafts as long as the donor and the

recipient were compatible at the B blood group locus. In order to be compatible, it was necessary that the donor of the skin not have any B antigen not found in the recipient. If grafts were made between chicks not compatible at the B locus, the grafts were rejected regardless of the compatibility of the other blood group loci.

Development of Cattle Blood Typing

Reagents used for determining chicken blood group loci in all studies cited above were made by immunizations within inbred lines. In the case of cattle, highly inbred lines are generally not available. Reagents are made by injecting red cells into unrelated cattle or rabbits. The resulting antiserum is made specific by removing unwanted antibodies by adsorption. Using this procedure, Ferguson (1941), Ferguson *et al.* (1942), and Stormont (1950) detected 40 different antigenic factors on cattle erythrocytes. The antigenic factors were given alphabetic symbols in the order of their detection: A, B, C, ...Z, and A', ...Z'. A number of additional antigenic factors have since been reported (Rendel, 1957). Stormont *et al.* (1951) reported studies of large sire families which demonstrated that at least 21 of 38 antigenic factors studied were controlled by multiple alleles at the B locus. Seven factors belonged to the C system. By 1952, Stormont (Rendel, 1957) had detected a minimum of 90 alleles in the B series. It was found that some alleles at the B locus give rise to antigens which have multiple specificities.

These antigenic factors are an integral part of the compound antigen and occur in several different, genetically determined arrangements.

Working with Friesian cattle in Holland, Bouw (1958) found that bulls carrying the Z gene had a progeny test for fat 0.059 ± 0.018 per cent higher than bulls without the Z. Rendel (1957) reported that the milk fat content in Swedish Red cows with the B allele BOY_2D' was 0.19 ± 0.03 per cent higher than for cows without the allele. Neimann-Sorensen and Robertson (1961) analyzed 2500 lactations from the Danish testing stations and found only one effect of statistical significance. This effect was the B allele BOY_1D' in Danish Red cattle where it increased the fat content of the milk by 0.064 ± 0.013 per cent. Robertson (1961) suggested from the Danish Red cattle data that not more than eight per cent of the genetic variance in fat content is controlled by the blood group loci. In the case of Rendel's Swedish Red cattle data, Robertson suggested that the B locus would control about eight per cent of the total variance and 16 per cent of the genetic variance.

Antigen Inheritance

Haldane (1937) and Irwin and Cole (1936) proposed that a single gene determines the presence of any one antigen. This one gene-one antigen concept postulates a simple one-to-one relationship between a specific gene and a specific antigen struc-

ture. Fisher proposed, in a suggestion presented by Race and Sanger (1958), that the human Rh antigens could be considered as the products of the action of a series of three pairs of very closely linked alleles, each gene in each pair being responsible for a single antigen with the ability to stimulate and react with only one kind of antibody. At the time that the Fisher-Race concept was established, antisera were known for four Rh antigens. Two additional antisera were predicted on the basis of this theory and one of these was later definitely established. The simplicity of this concept and the success of the prediction led to a wide acceptance of the Fisher-Race theory of closely linked genes.

Wiener and Landsteiner (1934) and Wiener (1944) presented a different genetic mechanism of inheritance of the Rh system. Wiener proposes that the Rh system is explained on the basis of multiple alleles at a single locus. One molecule of antigen may have different areas of specificity so that one antigen may combine with a number of antibodies of different specificities. This does not conflict with the one gene-one antigen concept. Either the Fisher-Race or Wiener systems can be used to describe the Rh antigens and antibodies. The National Institutes of Health require that commercial antisera be labeled according to both systems. The same problem arises in the human MNSs system. Here again Wiener (1948) explains this system on the basis of multiple alleles and Race and Sanger (1958) on the basis of very

closely linked genes. No crossovers have been noted in either system. To adherents of the Fisher-Race theory this attests to the closeness of the linkage. To the Wiener followers this challenges the linked gene theory. Stormont (1955) examined the theory of closely linked genes and of pseudoallelic genic elements as applied to blood groups. He suggested that the notion of closely linked genes and/or pseudoalleles arises from an erroneous impression that an antigen has a single specificity and gives rise to one specific kind of antibody. Stormont et al. (1951) found no evidence of allelism of individual antigenic factors in cattle. Instead, they reported that multiple allelism is shown by the groups of factors which are inherited as units. Landsteiner (1945) and Kabat and Mayer (1948) have shown that a single antigen can produce a multiplicity of antibodies.

Briles et al. (1950b) discussed the linked gene theory in connection with the A and B blood group systems in chickens. They preferred the multiple allele theory to explain their results. Gilmour (1959a) discussed these theories and expressed his belief that multiple allelism must be accepted. He reported that adsorption studies of B antisera indicate that a linked theory would necessitate the postulation of so many genes as to be an absurdity.

Irwin and Cole (1936) reported a "hybrid substance" on the red cells of species hybrids in doves. They showed that the hybrid cells possessed a specificity not present in either of

the parents. McGibbon (1944) found that species crosses between Mallard ducks and Muscovy contained a "hybrid substance" not present in either species. Miller (1956) found a "hybrid substance" in the cross between ring neck dove and domestic pigeon. He discussed a number of possible mechanisms that might result in this substance. Those discussed were: (a) subsurface position of substance in cells of parents; (b) an inhibitor present in one species; (c) rearrangement in the hybrid causing new specificities; (d) interaction of closely linked genes; and (e) allelic interaction resulting in a new substance. Cohen (1956, 1960) has on two occasions reported finding hybrid substance in rabbits.

Allen (1960) reported an iso-immune chicken antiserum that agglutinated the cells of some progeny but failed to agglutinate the cells of either parent. In the inbred line in which it was produced, the reagent was believed to be specific for the A locus. But in a second inbred line it agglutinated only certain B locus heterozygotes. When the antiserum was adsorbed with red cells from either parent, the antiserum still agglutinated red cells of the progeny.

METHODS AND MATERIALS

The Populations Used

The birds used in this study represented five lines of White Leghorns selected for single quantitative traits (Nordskog and Festing, 1962). In 1956, four commercial pure strains of White Leghorns were crossed reciprocally in a diallel set of matings. From the resulting offspring, four-way crosses were obtained in 1957. Since some matings were more prolific than others not all the original lines were represented equally in the second and subsequent generations after the initial cross. From the four-way crosses (second generation) five lines were selected as follows: A line, selected for high egg production from the first egg until early in December; B line, selected for large body size; C line, selected for small body size; D line, selected for large egg size; and E line, selected for small egg size. Selection of the large and small body lines was based on individual records. In the case of egg size, the females were selected on individual records and the males on a sib test. The egg production line was selected on an index taking into account the individual record, the dam family record, and the sire family record. Cockerels were selected on the basis of a sib test. These lines have been selected on the same basis for five years. During the first two years the A line was maintained in 16 single male pen matings with 14 selected females in each. The other

lines were propagated with eight single male pens with seven or eight females per pen. In the last three mating seasons, all mating pens have contained ten females each. An attempt has been made to raise enough birds so that those selected would represent approximately the upper 20 to 30 per cent of each population.

A restriction exercised in mating these lines was that no full or half sib matings were permitted. When several female progeny of a particular mating were selected they were systematically dispersed through the pens. This procedure was designed to minimize the amount of inbreeding.

The egg weights and body weights after five years of selection are summarized in Table 1. The figures given for B, C. D, and E lines are adjusted for year effects on the basis of A line. The body and egg weights were obtained in December. Had the weights been taken at a later date when the birds were more mature, the weights would have been somewhat heavier.

Table 1. Results of selection for body weight and egg weight

	Initial (1956)	Fifth generation (1961)				
		A	B	C	D	E
Egg wt. (grams)	57	57	63	50	64	47
Body wt. (lbs.)	4.3	4.3	6.3	3.2	4.6	3.6

Inspection of Table 1 reveals that selection for large body size resulted in an egg size nearly as large as that in the large egg line. Selection for small body size resulted in smaller egg size. Selection for egg size also changed body size. Thus, it is evident that primary selection for body or egg size not only changes the trait selected but also the secondary trait by a correlated response.

Egg production in the line selected for high egg production (A) has varied from year to year which is assumed to be due mainly to year to year environmental effects. The experimental results indicate that only slight improvement in egg production has been brought about by selection over the five years of selection in the A line. Because the four initial parent stocks were all commercial strains that had been selected for egg production for many years, it is possible that the rate of improvement in egg production has reached a plateau where little or no further improvement would be expected from the additional selection.

Basic Principles of Blood Typing

Blood antigens are protein-carbohydrate complexes on the surface of the red cell (Landsteiner, 1945). These antigens are controlled by a series of alleles at several loci. Injection of red cells of one individual into another gives rise to antibodies against those antigens that are foreign to the recipient. The antibodies produced may be specific for a particular antigen

under control of one allele. However, one antigen does not give rise to one specific kind of antibody. Instead, an antigen is thought to contain a number of chemical configurations, some of which may be antigenic in a particular recipient and give rise to an assortment of antibodies. This assortment, or array, is specific for the antigen, but some of the antibodies may also react with antigens controlled by a different allele at the same locus.

In this report the configurations that make up an antigen will be called antigenic determinants or antigenic factors. This corresponds to the procedure used in cattle blood typing. For example, Rendel (1957) cited earlier, dealt with a B allele in Swedish Red cattle designated BOY_2D' and Neimann-Sorensen and Robertson (1961) dealt with a B allele in Danish Red cattle designated BOY_1D'. These are considered to be two different alleles at the B locus. Each has four identified antigenic factors, three of which, B, O, and D' are in common to the two antigens. But because the Y_1 and Y_2 factors are different, the alleles are different.

The term antigen refers to the sum total of factors or determinants. Two factors having the same serological reactivity are generally considered to be identical. However, the possibility remains that two factors may be different yet have a sufficiently similar chemical nature so that their reactions would be similar.

Injecting red cells between members of the same species is called iso-immunization. An antiserum produced in this manner is an iso-immune serum. Production of an antiserum by injection of cells of one species into a different species is called hetero-immunization and the resulting antiserum is called hetero-immune serum. A reagent is an antiserum of known specificity. Greater specificity of an immune serum may be obtained by adsorption with red cells of some particular source. Antibodies in the antiserum react with specific antigens present on the surface of the cells. The cells separated from the fluid antiserum by centrifugation carry out the reacting antibodies. Thus a non-specific antiserum can be made into a highly specific reagent by removing all but certain antibodies.

In this study the antisera were modified by adsorption to reduce the spectrum of reactivity. Attempts were made to make reagents specific for single antigenic factors. The reagents are designated by a letter for the blood group locus followed by a number for the particular reagent involved. The first reagent for the B locus was designated B1, the second B2, etc. An allele controlling an antigen reacting with the reagents B1, B2, and B4 would be designated B_{1-2-4}. Two reagents giving the same results after appropriate testing are designated by the same number. Antigens present but for which no reagent is available are designated by the locus letter and a dash, such as B-. Cells failing to react may have antigens controlled by more than one allele in a multiple allele system, but are treated

as though only one antigen is present until additional definitive reagents become available.

Development of Reagents

The antisera used in this study were produced in rabbits and chickens. Hetero-immune sera were produced by injecting rabbits intravenously at seven day intervals with two ml of a 25 per cent suspension of chicken red cells. The same rabbit was always injected with cells from the same donor. An equal number of donors were used from each of the Leghorn lines used in this study. Ten days after the fifth injection, the rabbits were bled by cardiac puncture. Usually 60 to 80 ml of blood was withdrawn and citrated. The rabbits were again injected on a seven day schedule. They were bled a second time four weeks after the first bleeding. The citrated rabbit blood was centrifuged and the plasma portion withdrawn and heated to 56° C for 30 minutes to inactivate the complement. The fibrin was removed by centrifugation and discarded. The resulting antiserum was placed in an amber bottle, one drop of 1:1000 merthiolate was added per 20 ml of antiserum to prevent bacterial growth, and the antiserum was then stored in a freezer.

An attempt was made to produce hetero-immune antisera in turkeys, but resulted in failure because the turkeys died of anaphylactic shock before useful antiserum could be obtained.

Iso-immune antisera was produced in three ways. The first procedure involved injections between non-inbred full sibs. In

the second, injections were made between full sibs with inbreeding coefficients of .25. Both methods proved to be very satisfactory. In the third method which was less satisfactory, injections were made between individuals of two different lines. The immunization procedure was the same as with the rabbits except that the second bleeding was made three weeks after the first bleeding. The chicken blood was drawn by cardiac puncture using potassium oxalate as an anticoagulant. The chicken antisera were handled in the same manner as previously given for rabbit antisera.

All antisera were adsorbed. A preliminary series of adsorptions was made with each reagent to find a satisfactory method of adsorbing to increase the specificity of the antiserum. These trial adsorptions were made by mixing .5 ml of antiserum, two ml of physiological (.085) saline, and .5 ml of packed red cells. With high titer rabbit antiserum not previously adsorbed, it was usually necessary to repeat this step several times. The red cell-antiserum mixture was allowed to stand for 30 minutes before centrifuging and removing the supernatant for testing. The resulting adsorbed antisera was then tested with the red cells of 29 birds of which at least five birds were from each of the five lines of Leghorns used in this experiment. When adsorption successfully improved the specificity of an antiserum, the adsorption procedure was repeated using a larger volume of antiserum and a proportionately larger volume of red

cells. The resulting antiserum was again tested with 29 birds as before. Test adsorptions were again made with those cells among the 29 that were still agglutinated by the antiserum. If further test adsorptions indicated that the antiserum could be made still more specific, additional adsorptions were carried out. This procedure was continued until ultimately a trial adsorption made the antiserum inactive for all of the cells in the test. Occasionally, the adsorption procedure resulted in loss of all reactivity and the serum was discarded. Those sera suitable for use as reagents were identified by a number and stored in a freezer for future use. In all, 277 reagents were produced that appeared to be specific. Of these, 96 were of rabbit anti-chicken source and 181 were of chicken anti-chicken source. Before using these reagents in the testing carried out in this study, the reaction patterns of the various reagents were compared, in a test with cells of 89 birds from various lines. Several reagents gave identical test results. Some reagents that were nearly identical were adsorbed further in attempts to increase the specificity. The total number of adsorptions made in developing specific reagents exceeded 3,000.

For testing purposes, no more than four reagents were used that appeared to be identical. Where possible at least two identical reagents were used and run as pairs in an attempt to eliminate possible errors. Reagents low in quantity or titer

were not used. Initially 128 reagents were used to test the lines. Reagents giving indistinct results or which otherwise appeared to be unreliable as the testing proceeded were removed from the experiment. During the course of the experiments, 36 reagents were discarded. Ninety-two reagents were used throughout the study.

Testing Procedures

Birds to be tested were bled from a wing vein into a tube containing an anti-coagulant solution. This solution contained 20.5 grams glucose, eight grams sodium citrate, 4.2 grams of sodium chloride and sufficient distilled water to make a total volume of one liter. Later on, .1 ml of dihydrostreptomycin and one drop of 1:1000 Merthiolate were added to each liter to control bacterial growth. Test dilutions consisted of a two per cent red cell suspension in anti-coagulant solution. Tubes used for tests were 10 mm by 75 mm culture tubes. The test procedure consisted of placing .1 ml of appropriately diluted reagent in each tube. One drop of the two per cent cell solution was added to each tube. Tubes were agitated and allowed to stand for one hour. They were agitated a second time and allowed to stand for 30 minutes, and then agitated a third time and read after standing for one to two hours. Tubes were read by shaking gently in front of a narrow slit of light from a Kahn lamp and observing the degree of agglutination. Results

were recorded as negative, doubtful, or if positive, on a scale from one to six. Degree of agglutination is important for with some reagents one finds a dosage effect where homozygous (two doses) birds have a stronger agglutination than heterozygous (one dose). In the development of the reagents, more than 120,000 tube tests were run. In subsequent characterizations of the lines, and studying the inheritance of their blood groups, more than 110,000 additional tests were run.

It is possible in diluting reagents for testing, to make small errors in measuring. Such errors influence the strength of the agglutination reaction. If tests are made with a reagent that is too dilute, some weak reactions may not be observed and be classed as negative reactions. If reagent dilutions are too strong, some non-specific or low titer antibodies present in the reagent may cause weak positive reactions with cells that would otherwise be negative. If red cells are not properly handled the quality of their reactions may be adversely affected. Improperly washed tubes, moderately high laboratory temperatures, and bacterially contaminated diluents all contribute to experimental laboratory error.

In order to eliminate bias due to experimental error, not all the birds of one line were tested at one time. Instead, 44 birds of A line and 22 birds of each of the other lines were bled at one time. All of the cells from these birds were handled in the same manner and all tested at the same time with the

same reagent dilutions. Thus, any variation from one series of tests to another would have rather equal effects on each line.

Analysis of the Reagents

The reagents developed in this study were tested against birds of known blood group genotype. In addition to birds on the Iowa State University Poultry Farm, birds of six inbred commercial lines were tested.[1] The genotypes of the commercial lines were known for the A, B, C, D, E, L, X, and Z systems. The Iowa State University birds had not been blood typed for the E, X, or Z systems. The A and B systems originally reported by Briles et al. (1953) are probably not the same systems as those now designated A and B. With the exception of the X and Z systems, found by Allen[2] the systems involved in this study are believed to be the same as those described by Briles (1960) and Gilmour (1959b). After testing birds of known genotype the reagents fell into four categories: (1) clearly and consistently specific for one allele or antigenic factor, (2) specific for two identifiable alleles or factors in two different systems, (3) clearly specific but not for a known allele, and (4) erratic and inconsistent.

The Gene Frequency Sampling Problem

Gene frequencies in small populations change due to random drift (Wright, 1931). When a population is subdivided into

[1]Kindly furnished by Hy-Line Poultry Farms, Johnston, Iowa.

[2]Allen, Courtney. Johnston, Iowa. Studies of the X and Z systems. Private communication. 1961.

groups or lines, the lines represent a series of samples, each of two N genes, taken from the base population. The expected gene frequency of these samples, will be the same as that in the base population and the variance will be $\frac{q_o(1-q_o)}{2N'}$. The change of gene frequency (Δq) resulting from sampling in one generation can be stated in terms of its variance. This change in gene frequency due to sampling is called random drift. Its magnitude is influenced by the initial gene frequency (q_o) and the number of effective parents (N'). Effective parents are those parents that transmit gametes to the next generation. In ordinary polygamous domestic populations, the number of female parents are likely to be more numerous than the males. Thus, each male parent will have greater relative effect on the gamete complex determining the next generation, than will each female parent. The difference in numbers of the two sexes influences the effective number of parents according to the following formula (Wright, 1931):

$$1/2N' = (1/8M + 1/8F)\,[1-(1/8M + 1/8F)]$$

The M and F designate males and females, respectively. The second term of the formula decreases in importance as the number of parents increases. This formula also expresses the loss in heterozygosity due to inbreeding. The effective number of parents in a line can be calculated from the average number of effective sires and dams for each line. The effective number of sires and dams per generation for the five Leghorn lines used in this

study is shown in Table 2. The birds listed in any one generation are the progeny of the birds shown for the previous year.

Table 2. Effective number of sires and dams

Line	Parents	1956	1957	1958	1959	1960	1961
A	sires	12	9	11	10	12	15
	dams	29	30	35	35	39	56
B	sires	13	8	8	8	9	8
	dams	21	18	23	28	28	43
C	sires	15	8	8	8	9	8
	dams	22	15	20	26	30	41
D	sires	14	8	8	8	8	8
	dams	19	15	23	25	33	44
E	sires	13	7	8	9	7	8
	dams	17	15	16	22	23	39

The mean number of sires and dams and the computed effective number of parents is shown in Table 3. The number of parents shown in Table 2 for years 1956 and 1961 were not used in calculating the effective number of parents. The 1956 parents were not selected for quantitative traits and may have been parents of 1957 birds in several different lines. The birds listed as 1961 parents were parents of birds selected for breeders in 1962, but some of the 1962 breeders may not have

produced offspring. Omitting these two generations may give an underestimated effective number of parents. However, this is more desirable than a possible overestimation.

Table 3. Effective number of parents

Line	Mean number of sires	Mean number of dams	Effective number of parents
A	11	34	36
B	8	27	25
C	8	23	25
D	8	24	25
E	8	19	24

The differences in blood group frequencies between the lines used in this study were tested for statistical significance by the following procedure. First, gene frequencies were estimated for each line. Secondly, from the effective number of parents (N') the variance of the gene frequency (s_q^2) was estimated. Thirdly, the 95 per cent confidence interval for each gene frequency estimate was obtained by setting upper and lower limits equal to $(1.96)(s_q)$ from the estimated base population gene frequency ($\hat{q}_o$).

Since the present investigation was undertaken some years after the lines were developed, the question arises as to the best estimate of the base population gene frequency, q_o. One possibility would be the overall average of the lines selected

for traits in two directions as and estimate of q_o. But here the question is whether selection for a given trait had been equally effective in the upward direction as in the downward direction. The second alternative, and the one chosen, was to use the gene frequency of the egg production line (A) as a reasonable estimate of q_o. The A line has been maintained with twice as many selected breeders as any other one line. This line appears not to have responded to selection for rate of production, indicating that a plateau has been reached and that selection for egg production did not cause much genetic change in the population. The A line has not changed appreciably in body size or egg size, indicating that the genes for these traits are probably close to an equilibrium state. Any selection for body or egg size in the original commercial lines would probably have been for a moderation of body and egg size as extreme body or egg size is not considered desirable in commercial poultry. The procedure for setting 95 per cent confidence intervals is given in Mood (1950) for the single parameter p, of a binomial distribution

$$P[\hat{p} - 1.96\sqrt{\frac{p(1-p)}{n}}] < p < [p + 1.96\sqrt{\frac{p(1-p)}{n}}]$$

where p is the parameter estimated with variance

$$\sigma_p^2 = \frac{p(1-p)}{n}$$

Using the formula (Crow, 1954) for variance due to random drift in t generations,

$$\sigma^2 = (q)(1-q)\left[1 - \left(1 - \frac{1}{2N}\right)^t\right]$$

or,

$$\sigma^2 = (q)(1-q)\,[K]$$

and substituting in Mood's the following expression for estimating the confidence intervals due to random drift is obtained:

$$q_{o_L} = \frac{\frac{2qt}{K} + (1.96)^2 - 1.96\sqrt{\frac{4qt(1-qt)}{K} + (1.96)^2}}{2\left[\frac{1}{K} + (1.96)^2\right]} = \text{lower limit}$$

$$q_{o_U} = \frac{\frac{2qt}{K} + (1.96)^2 + 1.96\sqrt{\frac{4qt(1-qt)}{K} + (1.96)^2}}{2\left[\frac{1}{K} + (1.96)^2\right]} = \text{upper limit}$$

where q_o is the initial gene frequency, qt is the gene frequency in the $\underline{t}$ generation, and $K = \left[1 - \left(1 - \frac{1}{2N}\right)^t\right]$.

Validity of these formulae is based on the following assumptions: (1) no migration, (2) no mutation, (3) generations do not overlap, (4) number of breeding individuals in each line are the same for all lines and in each generation, and (5) mating is random. In the present study these conditions would seem to be reasonably well satisfied. All Leghorn lines are closed flocks with pedigreed matings. Mutation rates are expected to be so low that this should not be of any consequence in only five years. Effective selection in these lines has been only for body and egg size. The blood group genes of concern in this study have not been directly subjected to selection. Matings were made at approximately the same time each year so

that generations did not overlap and ages were approximately the same from year to year.

The number of birds mated in the four lines, B, C, D, and E, where drift is of interest, had the same number of pens, males, and females each year. The effective number of parents varied very slightly from line to line. The departure from random breeding in the breeding plans used with these lines was such as to reduce the expected inbreeding. The formula for the effective number of parents also expresses the loss of heterozygosity due to chance inbreeding. Since the amount of inbreeding was less than would occur with random mating, this would tend to underestimate the effective number of parents. This would decrease the chance of a "type I" error in the conclusions.

Comparisons were made between the estimated confidence intervals due to random drift for the initial gene frequency of two lines. Since the lines originated from the same base population with initial gene frequency q_0, any difference in observed gene frequency between two lines due to random drift would be expected to bracket the original gene frequency q_0. Three possible conditions may exist in such comparison. These are illustrated in Figure 1.

Comparisons between lines are based on the null hypothesis that differences are solely due to random drift. If two confidence intervals overlap the null hypothesis is accepted. If they do not overlap the null hypothesis is rejected. The alterna-

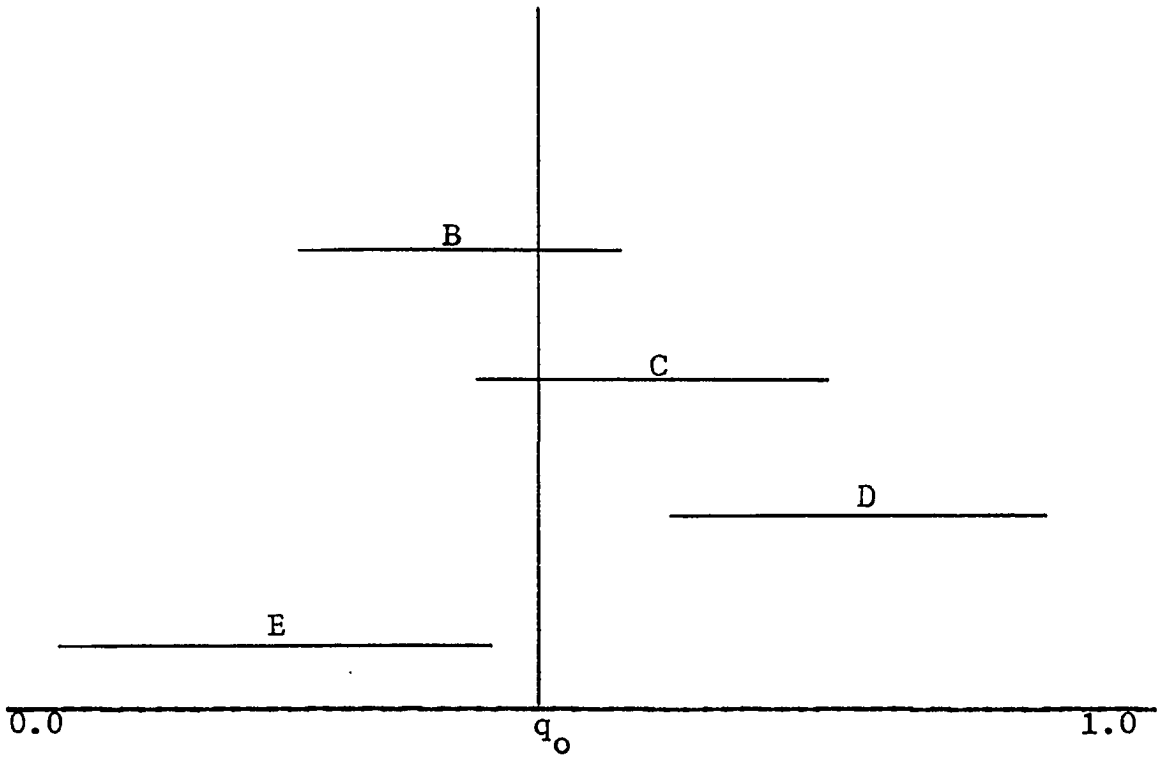

Figure 1. Confidence intervals in relation to initial gene frequency

tive hypothesis is that random drift alone does not account for the differences between lines.

If the q_o does not fall within the confidence interval, as in the case of lines D and E in Figure 1, then comparisons of these lines would lead to a correct rejection of the null hypothesis. If the confidence intervals overlap as in lines B and D, the null hypothesis would be falsely accepted leading to a type two error.

If we compare line B and line C, q_o is within both confidence intervals and random drift could account for any observed differences between the gene frequencies in generation t. In comparing lines D and E, q_o does not fall in either confidence interval. In this case the differences between the two lines cannot be as easily explained on the basis of random drift because q_o would not fall in both confidence intervals.

In order to test the null hypothesis, we construct a $(1-\alpha)$ 100 per cent confidence interval on q_o for each line. In order to set a five per cent probability of a type one error, we choose α such that $2(\alpha/2)^2 = .05$, or $\alpha = \sqrt{.1}$, or .3162. In the formulae for confidence intervals $\alpha/x = 1.96$. This requires correction to reflect $\alpha = .3162$ or $\alpha/x = 1.002$. Finally the formulae for testing the hypothesis is:

$$q_o = \frac{\frac{2qt}{K} + (1.002)^2 \pm 1.002\sqrt{\frac{4qt(1-qt)}{K} + (1.002)^2}}{2\left[\frac{1}{K} + (1.002)^2\right]}$$

RESULTS

Characterization of the Lines

The adult birds selected for breeders in 1962 were tested with 92 reagents. Seventy of these identified 19 antigenic factors or alleles of known systems, 12 were specific for 12 factors or antigens of unidentified systems, and ten contained antibodies for more than one system. The number of birds tested in each line is shown in Table 4. The resulting phenotypes, based on reactions to these reagents are presented in Appendix A.

Table 4. Number of birds blood typed

Line	Number of birds	Trait selected
A	172	High egg production
B	88	Large body
C	87	Small body
D	86	Large egg
E	87	Small egg

The B blood group system contains a great many alleles each with a number of antigenic factors. The phenotypes listed for the B system are the complement of antigenic factors of the B alleles present in each bird. These phenotypes were assigned on the basis of reactions to reagents for 13 different B factors. Phenotypes that now appear identical might be further differen-

tiated if more B reagents were available. The B system alleles that can be postulated on the basis of breeding tests (Tables 19, 20, and 21) are presented in Table 5.

Table 5. Postulated B system alleles

Alleles	Alleles
$B_{2-3-7-13}$	B_9
B_{6-9}	B_{9-14}
B_{6-12}	B_{10-11}
$B_{6-12-14}$	B_{10-14}
B_{6-7}	B_{11-13}
B_7	B_{12}
B_{7-12}	B_{14}
B_{7-13}	

A number of factors are components of more than one allele. The blood group systems other than B appear to have few alleles. The A, X, and Z systems appear to contain few alleles. Only one reagent was available for each system. If more than two alleles exist in the population tested, the frequencies would be small as each of the alleles identified in the A, X. and Z systems has a rather high gene frequency (Table 11).

The E system must have at least three alleles, as two alleles were identified and a number of birds had neither of these.

The gene frequencies of these two alleles taken together account for only one half of the total for the system.

Only one D allele with a total gene frequency for 5 lines of .13 was identified. Several more D alleles may be present.

Differences between Lines

Differences were found between lines in reactivity to some of the reagents. The gene frequencies[1] of each line for all reagents, except those for the B system, were calculated. Since the gene frequencies of the A line were used to estimate the base population (q_o), the gene frequencies of the other lines were all compared to the A line. Gene frequency estimates are presented in Table 5 for line B, Table 6 for line C, Table 7 for line D, and Table 8 for line E. Confidence intervals at the 95 per cent level are also included in the tables.

The gene frequencies of A line are compared with the mean gene frequencies of the five lines and presented in Table 10. The results show the two sets of estimates to be in close agree-

[1]The alleles A_1, D_1, E_1, E_2, X_1, and Z_1 are so designated because they are specific for alleles of these systems in seven inbred lines, and in the case of D_1, X_1, and Z_1 segregate independently in the non-inbred population being tested. Eight reagents reacting with antigens that appear to be segregating independently in this non-inbred population, but not identified as to system with seven inbred lines, are treated here as alleles. One or more of these eight reagents may ultimately prove to be specific for antigenic factors instead of alleles. However, with the information available, treatment of antigens reacting to these reagents as alleles seems justified for the purposes of this study.

Table 6. Estimated gene frequencies and confidence intervals for B line

Reagent	95% conf. intervals lower	upper	Initial gene frequency ($\hat{q}_o$)	Gene frequency ($\hat{q}_t$)
A1	.40	.97	.69	.39*
D_1	.00	.09	.02	.12*
E_1	.01	.58	.29	.25
E_2	.00	.54	.27	.39
X_1	.40	.97	.69	.52
Z_1	.06	.65	.35	.37
69	.00	.52	.25	.27
82	.04	.07	.06	.05
133	.00	.07	.06	.04
141	.03	.05	.04	.11*
142	.05	.08	.07	.11*
145	.07	.10	.09	.13*
169	.06	.10	.09	.13*
170	.12	.16	.14	.20*
171	.03	.05	.04	.04

*Estimated gene frequency is outside confidence intervals

Table 7. Estimated gene frequencies and confidence intervals for C line

Reagent	95% conf. intervals lower	upper	Initial gene frequency ($\hat{q}_o$)	Gene frequency ($\hat{q}_t$)
A_1	.40	.97	.69	.43
B_1	.00	.09	.02	.04
E_1	.01	.58	.29	.01*
E_2	.00	.54	.27	.30
X_1	.41	.97	.69	.72
Z_1	.06	.65	.35	.26
69	.00	.52	.25	.20
82	.04	.07	.06	.01*
133	.04	.07	.06	.01*
141	.03	.05	.04	.03
142	.05	.08	.07	.02*
145	.07	.10	.09	.04*
169	.06	.10	.08	.10*
170	.12	.16	.14	.12
171	.03	.05	.04	.01*

*Estimated gene frequency is outside confidence intervals

Table 8. Estimated gene frequencies and confidence intervals for D line

Reagent	95% conf. intervals lower	95% conf. intervals upper	Initial gene frequency ($\hat{q}_o$)	Gene frequency ($\hat{q}_t$)
A_1	.40	.97	.69	.43
D_1	.00	.09	.02	.35*
E_1	.01	.58	.29	.29
E_2	.00	.54	.27	.35
X_1	.41	.97	.69	.71
Z_1	.06	.65	.35	.38
69	.00	.52	.25	.16
82	.04	.07	.06	.10*
133	.04	.07	.06	.07
141	.03	.05	.04	.15*
142	.05	.08	.07	.22*
145	.07	.10	.09	.21
169	.06	.10	.08	.21*
170	.12	.16	.14	.17*
171	.03	.05	.04	.08*

*Estimated gene frequency is outside confidence intervals

Table 9. Estimated gene frequencies and confidence intervals for E line

Reagent	95% conf. intervals lower	95% conf. intervals upper	Initial gene frequency ($\hat{q}_o$)	Gene frequency ($\hat{q}_t$)
A_1	.39	.98	.69	.85
D_1	.00	.09	.02	.12*
E_1	.01	.58	.29	.26
E_2	.00	.55	.27	.28
X_1	.40	.98	.69	.72
Z_1	.05	.66	.35	.36
69	.00	.52	.25	.33
82	.00	.20	.06	.05
133	.00	.20	.06	.01
141	.00	.16	.04	.02
142	.00	.22	.07	.07
145	.00	.26	.09	.17
169	.00	.20	.08	.12
170	.00	.45	.14	.22
171	.00	.17	.04	.09

*Estimated gene frequency is outside confidence intervals

ment. In no case do the gene frequencies of line A fall outside of the 95 per cent random drift confidence intervals.

A number of gene frequencies presented in Tables 6, 7, 8, and 9 are greater or less than would be expected 95 per cent of the time due to random drift. Significant differences are found for seven alleles in B line, seven alleles for C line, eight alleles in D line, and one allele in E line. Those alleles falling outside of the 95 per cent confidence limits for random drift may be due to forces other than random drift such as mutation, migration, and selection.

The gene frequencies are presented again in summary form in Table 11. Comparisons of interest are B line with C line and D line with E line wherein selections were made in opposite directions for body weight and egg weight, respectively. In the case of the B and C lines, seven gene frequencies fall outside the confidence intervals. Only three of these involve the same reagents. Of these, numbers 142 and 145 are outside the confidence intervals at opposite extremes.

In comparing egg weight lines only one gene frequency is outside the random drift confidence interval in the E line while eight D line gene frequencies are outside the confidence intervals. The computed confidence intervals are larger for E line than for the other lines due to a slightly smaller effective number of parents.

Table 10. Estimated gene frequencies and confidence intervals for A line

Reagent	95% conf. interval lower	95% conf. interval upper	Mean gene frequency all lines ($\bar{q}_t$)	Gene frequency ($\hat{q}_t$)
A_1	.31	.80	.56	.59
D_1	.00	.29	.13	.02
E_1	.05	.39	.22	.29
E_2	.07	.52	.30	.27
X_1	.44	.90	.67	.69
Z_1	.11	.58	.34	.35
69	.03	.48	.24	.25
82	.00	.17	.05	.06
133	.00	.16	.04	.06
141	.00	.21	.07	.04
142	.00	.24	.10	.07
145	.00	.27	.11	.09
169	.00	.29	.13	.09
170	.00	.36	.17	.14
171	.00	.16	.05	.04

Table 11. Summary of gene frequency estimations for five lines

Reagent	A	B	C	D	E
A_1	.69	.39*	.43	.43	.85
D_1	.02	.12*	.04	.35*	.12*
E_1	.29	.25	.01*	.29	.26
E_2	.27	.39	.30	.35	.18
X_1	.69	.52	.72	.72	.72
Z_1	.35	.37	.26	.38	.36
69	.25	.27	.20	.16	.33
82	.06	.05	.01*	.10	.05
133	.06	.04	.01*	.07	.01
141	.04	.11*	.03	.15*	.02
142	.07	.11	.02	.22*	.07
145	.09	.13*	.10*	.21*	.12
170	.14	.20*	.12	.17*	.22
171	.04	.04	.01*	.09*	.09

*Outside random drift confidence intervals

Correlated Responses

The gene frequency arrays are similar between B and D lines. This could be due to correlated responses to selection. Thus selection for large body size in B line has evidently increased egg size as a correlated response to the direct effect of selection in the D line.

If egg size and the blood groups have genes in common, or if they are linked, then lines similar in egg size and body size would also be similar in blood groups. The agreement between the blood group gene frequencies of B and D lines is rather close with six common gene frequencies outside the random drift confidence intervals. The gene frequencies of C and E also show similarity. Three alleles, A_1, D_1, and E_1, and reagents for eight unknown systems (Table 11) were found to have gene frequencies outside the random drift confidence intervals. Correlations were computed between each of the lines for these eleven gene frequencies. These correlations are presented in Table 12.

Table 12. Correlations between lines for gene frequencies

	E	D	C	B
A	.98	.70	.88	.92
B	.91	.82	.84	
C	.94	.67		
D	.76			

If correlated responses exist between blood groups and egg or body size, those lines that are similar, (B vs. D and C vs. H,)should have higher correlations than those lines that are different (B vs. C and D vs. E). The correlations between B and D (.92) and C and E (.94) are higher than the correlations

between B and C (.84) and D and E (.76). However, all correlations are high.

Since the A line seems likely not to have changed due to selection for egg production, partial correlations were computed between the lines from the correlations given in Table 12, holding the A line gene frequencies constant. Only two of these partial correlations, presented in Table 13, are statistically significant. The others are all positive but much smaller.

Table 13. Partial correlations with A line constant

Correlations
BC.A = .09
BD.A = .63*
BE.A = .19
CD.A = .14
CE.A = .76**
DE.A = .45
N = 11, df = 8

* .05
** .01

Comparisons were made between each combination of lines for confidence intervals on the original gene frequency, q_o, computed from the estimated gene frequency in the fifth gen-

eration, q_t. These comparisons were made between each of the lines for the five designated blood group systems and each of the eight reagents for unidentified systems. The comparisons where differences occurred that could not be explained on the basis of random drift alone are shown in Table 14.

Table 14a. Line comparisons for differences assumed not to be due to random drift

Reagent	Significant comparisons
A_1	A-B, A-C, A-D, B-E, C-E, D-E
D_1	A-D, B-D, C-D, D-E
E_1	A-C, B-C, C-D, C-E

Comparisons were made between the gene frequencies of the lines for individual alleles, as determined by reactions to blood typing reagents, and the differences were tested by a studentized t test,

$$t = \frac{q_1 - q_2}{(q_o)(1-q_o)[K_A+K_B]}$$

where q_o is the initial gene frequency as estimated from the A line and $K = 1 - (\frac{1}{2N})^6$ is a correction factor for effective population number, N, and the number of selections made.

The results are presented in Table 14b. Three line differences with respect to the A, allele were significant at the five per cent level: B-E, C-E, and D-E. For the D_1 allele four line differences were significant: A-D, B-D, C-D, and D-E. All of these were significant at the one per cent level.

Considerable similarity of results were obtained using the A line to estimate q_o (method one) as given in Table 14b, compared with method two where comparisons were made between calculated initial gene frequency confidence intervals. Method two did not require estimates of q_o. Of the 14 significant differences found by method two (Table 14b), method one (Table 14a) indicated four of these to be significant at the one per cent level, three at the five per cent level, and the other seven at levels between five and ten per cent.

The most important objection in using the t test of differences between lines concerns the use of the A line to estimate the initial gene frequency in the base population. However, the close agreement between results of methods one and two can be considered further indication of the adequacy of the A line to estimate the initial gene frequencies.

Comparisons that have been made between lines for gene frequencies do not include B system reagents. The B reagents are specific for antigenic factors and are components of a number of alleles. If all of the antigenic factors for the B system alleles were known and the reagents available, the B system alleles could be perfectly characterized and studied. The thirteen B system reagents used in this study are probably inadequate to fully characterize all of the alleles existing in the population. In several lines, particularly in the B line, several birds demonstrated few positive reactions for the B reagents. This is assumed to be a consequence of insufficient numbers of reagents to characterize all of the

Table 14b. The *t* tests of significance for gene frequency comparisons between lines

Reagent	Comparison									
	A-B	A-C	A-D	A-E	B-C	B-D	B-E	C-D	C-E	D-E
A_1	1.50	1.26	1.28	.83	.21	.18	2.28	.03	2.08*	2.10*
D_1	1.84	.36	6.15**	1.95	1.47	4.31**	.07	5.78**	1.58	4.20**
E_1	.24	1.55	.05	.20	1.31	.19	.04	1.52	1.36	.15
82	.11	.46	.47	.11	.36	.58	.00	.93	.36	.58
133	.17	.50	.09	.46	.37	.26	.39	.59	.04	.55
141	.96	.10	1.43	.25	1.06	.34	1.21	1.53	.18	1.68
142	.44	.40	1.42	.01	.82	.98	.45	1.81	.38	1.42
145	.35	.36	.49	.71	.72	.14	.36	.86	1.08	.22
169	.44	.18	1.06	.33	1.09	.62	.11	.88	.15	.73
170	.41	.12	.21	.56	.53	.27	.14	.33	.67	.35
171	.08	.38	.60	.60	.62	.68	.68	.98	.98	.00

*p .05

**p .01

alleles. To designate the B system alleles or try to calculate gene frequencies would therefore seem premature. The distribution of these antigenic factors may be examined even though allele information is incomplete. The frequency of positive reactions to the B reagents are shown in Table 15. Definite differences exist between the lines. Line C has a much higher proportion of reactions than the other lines for reagents B1, B2, B3, and B5. Birds in C line that are positive to B2 are usually positive to B3. A bird positive to B1 is usually positive to both B2 and B3. The B5 factor also seems to be associated with B2 and B3, but not necessarily with B1. B1, B2, and B3 may be antigenic factors in one allele and B2, B3, and B5 factors in another allele. These combinations are much more numerous in C line than in the other lines.

In D line, 49 per cent of the birds were positive to reagent B11. This is twice the number in A line, four times the number in B line, seven times the number in C line, and eight times the number in E line. Obviously D line is different from the other lines at the B locus. This is evident even though the alleles involved are not known. The lack of knowledge concerning the alleles in the B system makes speculation in regard to these differences hazardous. However, the obvious differences would indicate the need for further research on the B system alleles.

Correlations between each of the lines for per cent posi-

Table 15. Frequency of positive reactions to B reagents

Reagent	A	B	C	D	E
B_1	.05	.02	.11	.03	.08
B_2	.02	.09	.45	.28	.10
B_3	.07	.09	.47	.24	.09
B_4	.41	.41	.22	.20	.59
B_5	.01	.03	.10	.06	.00
B_6	.60	.41	.77	.60	.51
B_7	.69	.76	.51	.41	.85
B_9	.38	.32	.28	.29	.20
B_{10}	.12	.14	.00	.13	.01
B_{11}	.22	.11	.07	.49	.06
B_{12}	.38	.45	.62	.60	.48
B_{13}	.06	.33	.44	.17	.05
B_{14}	.50	.30	.23	.38	.32

tive reactions for each of the B reagents are presented in Table 16. Partial correlations were computed between the lines from the correlations presented in Table 16, holding A line constant. These partial correlations appear in Table 17.

The only statistically significant partial correlation in Table 17 is between B and E. The reason for this significance is not known. Little importance can be attached to these correlations as they represent correlations between antigenic

Table 16. Correlations between lines for antigenic factors

	E	D	C	B
A	.89	.68	.45	.88
B	.92	.54	.56	
C	.54	.64		
D	.55			

Table 17. Partial correlations with A line constant

Correlations
BC.A = .37
BD.A = -.02
BE.A = .64*
CD.A = .50
CE.A = .03
DE.A = -.18

n = 13, df = 10, $p < .05$

factors and not genetic materials. The antigenic factors in one line may be controlled by entirely different alleles than the same antigenic factors in another line.

Heritable Nature of Blood Group Systems

A "repeat mating" system was used with the A line for estimating yearly environmental effects (Goodwin et al., 1960). This involves holding over certain birds for a second breeding season. In the winter of 1961-62 two generations of A line breeding birds were available for blood group testing. These included the regular matings of the five lines plus some A line matings used in 1961 retained for repeat mating controls. Progeny of both groups were available for testing. The progeny produced by the 1962 repeat matings were non-contemporary sibs of some 1962 A line selected breeders. The progeny of the regular matings represented a third generation. The genetic segregation of the reactions to the different reagents were studied in these three generations.

The A-E System

The A and E systems are considered jointly because evidence indicates they are closely linked. The matings studied and the segregation of offspring from these matings are shown in Table 18. The close linkage reported in the literature is also evident in this study. Based on the postulated genotypes of the parents, the phenotypes of the progeny are as expected with complete linkage of the A and E systems. The genotypes of the offspring were not determined.

Table 18. Genetic segregation of the A-E system

Parents				Offspring			
Sire		Dam		Number of each phenotype			
Phenotype	Genotype	Phenotype	Genotype	A_1 E_1	A_1E_2	A_1 E_1 E_2	A- E_2
$A_1E_1E_2$	$\frac{A_1E_1}{A\text{-}E_2}$	A- E_2	$\frac{A\text{-}\ E_2}{A\text{-}\ E\text{-}}$	1	0	6	5
		A_1E-	$\frac{A_1\ E\text{-}}{A\text{-}\ E\text{-}}$	6	0	0	4
		A_1 E_2	$\frac{A_1\ E\text{-}}{A\text{-}\ E_2}$	9	8	4	6
		$A_1E_1E_2$	$\frac{A_1\ E_1}{A\text{-}\ E_2}$	4	0	6	3
		A- E_2	$\frac{A\text{-}\ E_2}{A\text{-}\ E_2}$	0	0	2	3
		A_1 E_1	$\frac{A_1\ E_1}{\ \ \ \ }$	4	0	0	2
A_1 E_1	$\frac{A_1\ E_1}{A_1\ E_1}$	A_1 E-	$\frac{A_1\ E\text{-}}{A_1\ E\text{-}}$	14	0	0	0
		A_1 E_2	$\frac{A_1\ E\text{-}}{A\text{-}\ E_2}$	6	0	10	0
		A_1 E_1E_2	$\frac{A_1\ E\text{-}}{A\text{-}\ E_2}$	7	0	5	0
		A_1 E_1	$\frac{A_1\ E_1}{A\text{-}\ E\text{-}}$	21	0	0	0

The B System

The B system contains a large number of alleles. Male H5046 reacted with B reagents B6, B7, B12, and B14. His phenotype is designated $B_{6-7-12-14}$. The sire and dam of this male were B_{4-7-12} and $B_{6-12-14}$, respectively. Fourteen full sibs of male H5064 were B_{4-7-12} and eleven were $B_{4-6-12-14}$. The sire's genotype is postulated to be $\frac{B4-12}{B7}$ and the dam's genotype $\frac{B6-12-14}{B6-12-14}$. The full sibs are $\frac{B7}{B6-12-14}$ and $\frac{B4-12}{B6-12-14}$. Male H5046 was postulated to have the genotype $\frac{B7}{B6-12-14}$. The results of mating male H5046 to six females whose genotypes were postulated in a similar manner is shown in Table 19. In Tables 20 and 21, results of matings with males H5170 and H3396 are presented. The genotypes of these matings were deduced in a similar manner to male H5046.

The D System

Only one D system reagent was available. This reagent was unusual because the agglutination process was very slow. Agglutination tests read in less time than two hours after mixing gave very weak positive reactions that might be mistaken for no reaction. If the tubes were allowed to incubate for three or four hours after mixing cells with reagent the agglutination was extremely strong and positive reactions were very easy to determine. The test results could not always be

Table 19. B blood group phenotypes of progeny of male H5064

Sire genotype	Dam genotype	Offspring phenotype	Number
$\frac{B_7}{B_{6}\text{-}12\text{-}14}$	$\frac{B_7}{B_{6}\text{-}9}$	$B_{6}\text{-}9\text{-}12\text{-}14$	3
		$B_{6}\text{-}7\text{-}12\text{-}14$	4
		B_7	1
		$B_{6}\text{-}7\text{-}9$	2
	$\frac{B_{6}\text{-}9}{B_{7}\text{-}13}$	$B_{6}\text{-}9\text{-}12\text{-}14$	3
		$B_{6}\text{-}7\text{-}9$	2
		$B_{7}\text{-}13$	2
	$\frac{B_{6}\text{-}12}{B_{7}\text{-}13}$	$B_{6}\text{-}7\text{-}12\text{-}13\text{-}14$	3
		$B_{7}\text{-}13$	2
	$\frac{B_{6}\text{-}12}{B_{7}\text{-}13}$	$B_{6}\text{-}7\text{-}12\text{-}13\text{-}14$	4
		$B_{6}\text{-}12\text{-}14$	2
		$B_{6}\text{-}7\text{-}12$	2
	$\frac{B_{6}\text{-}9}{B_{11}\text{-}13}$	$B_{6}\text{-}9\text{-}12\text{-}14$	1
		$B_{7}\text{-}11\text{-}13$	2
	$\frac{B_{7}\text{-}}{B_{6}\text{-}9}$	$B_{6}\text{-}7\text{-}12\text{-}14$	3
		$B_{6}\text{-}7\text{-}9$	3
		$B_{6}\text{-}9\text{-}12\text{-}14$	4
		B_7	1

Table 20. B blood group phenotype of offspring of male H5170

Sire genotype	Dam genotype	Offspring phenotype	Number
$\frac{B_{6-9}}{B_{10-11}}$	$\frac{B_{6-7}}{B-}$	B_{10-11}	2
		$B_{6-7-10-11}$	1
		B_{6-7-9}	1
	$\frac{B_{6-9}}{B_{14}}$	$B_{6-9-10-11}$	5
		B_{6-9}	1
		B_{6-9-14}	1
	$\frac{B_{6-7}}{B_{12}}$	B_{6-9-12}	1
		$B_{6-7-10-11}$	1
		$B_{10-11-12}$	3
		B_{6-7-9}	1
	$\frac{B_{2-3-7-13}}{B_{6}}$	$B_{2-3-7-13-10-11}$	4
		$B_{6-10-11}$	3
		$B_{2-3-6-7-9-13}$	3
		B_{6-9}	3
	$\frac{B_{6-7}}{B_{9}}$	B_{6-7-9}	1
		$B_{9-10-11}$	1

Table 21. B blood group phenotype of offspring of male H3396

Sire genotype	Dam genotype	Offspring genotype	Number
$\frac{B_7}{B_{10-14}}$	$\frac{B_{6-7}}{B_{9-14}}$	$B_{9-10-14}$	3
		B_{7-9-14}	3
	$\frac{B_{6-9}}{B_{10-14}}$	B_{10-14}	1
		$B_{6-9-10-14}$	4
		B_{6-7-9}	2
	$\frac{B_{7-12}}{B_{14}}$	B_{10-14}	1
		B_{7-12}	5
		$B_{7-10-12-14}$	2
		B_{7-14}	1

read when maximum accuracy would be achieved. Errors due to reading before reactions had become strong, if any, would tend to reduce the number of positive reactions. The results of the test of inheritance for this reagent are shown in Table 22. These genetic segregations are not significantly different from expected ratios as indicated by small chi-square values.

Table 22. Genetic segregations of reactions for the D system

Sire genotype	Dam genotype	Progeny phenotypes D-	D_1	χ^2
D- / D-	D- / D-	178	0	0
D- / D-	D_1 / D-	12	17	.86
D_1 / D-	D- / D-	39	29	1.47

The X System

Only two types of matings were found in the lines studied. The matings and results are shown in Table 23. The chi-square values indicate that the ratios of phenotypes do not differ significantly from the expected ratios for a single allele.

Table 23. Genetic segregation of reactions for the X system

Sire genotype	Dam genotype	Progeny phenotype X_1	X-	χ^2
X_1 / X_1	X_1 / X_1 or X_1 / X-	122	0	0
X_1 / X-	X_1 / X-	68	22	.79

The Z System

The segregation of the Z system is shown in Table 24. The segregations are reasonable expectations of mendelian ratios for a single gene as indicated by non-significant chi-square values.

Table 24. Genetic segregation of reactions for the Z system

Sire genotype	Dam genotype	Progeny phenotypes Z_1	Progeny phenotypes Z-	χ^2
$\frac{Z_1}{Z_1}$	$\frac{Z_1}{Z_1 \text{ or } Z-}$	76	0	0
$\frac{Z_1}{Z-}$	$\frac{Z_1}{Z-}$	107	38	.32
$\frac{Z_1}{Z-}$	$\frac{Z-}{Z-}$	35	28	.79

Other Systems

Eight tested reagents could not be identified with any specific blood group system. They segregated independently of known blood group systems in seven inbred lines of known genotype. Reactions in the population studied are shown in Table 25. The results indicate that the ratios are essentially mendelian. None of the chi-square values are statistically significant. However, in the case of reagent no. 142, not

Table 25. Segregation of responses to reagents for undetermined systems

Reagent	Sire genotype	Dam genotype	Progeny phenotypes (+)	(-)	χ^2
69	+ / -	+ / -	28	14	1.56
	+ / -	- / -	69	89	2.53
	- / -	- / -	0	59	0
82	+ / -	- / -	49	59	.93
	- / -	- / -	4	127	-
133	+ / -	- / -	27	26	.02
	- / -	- / -	4	127	-
141	+ / -	- / -	18	23	.61
	- / -	- / -	1	238	-
142	+ / -	- / -	2	1	-
	- / -	- / -	0	269	0
145	+ / -	- / -	4	6	.20
	- / -	- / -	0	242	0
169	+ / -	- / -	11	7	.44
	- / -	- / -	0	200	0
170	+ / -	- / -	115	136	1.76
171	+ / -	- / -	90	79	.716
	- / -	- / -	0	22	0

enough critical test offspring were available for a reliable test. In several cases a few positive reactions appeared among progeny of two parents classified as homozygous negative. These cases probably represent error in the classification of parent genotype or the phenotype of the progeny. Three unexpected reactions were found in the progeny of birds classified as homozygous negative for reagent no. 169. All three individuals were the progeny of one dam. Re-tests disclosed that the dam had been incorrectly classified as negative to reagent no. 169. A re-test of the birds involved in the unexpected positive reactions among progeny of homozygous negative matings for reagents 82, 133, and 141 was not possible.

DISCUSSION

Eighty-nine of 92 reagents used in this study identified blood group antigens that segregate in a manner expected for single genes. This indicates that the procedures used are sound and of value in characterizing a non-inbred population. On the other hand, the techniques used by Fanguy (1958) in testing a non-inbred population, using "broad spectrum reagents" developed in inbred lines, would seem to have considerable limitations.

The results obtained for the B system in this study probably could not have been obtained with Fanguy's techniques unless an extremely large number of different reagents were available. Such a number would be impractical, if not impossible. Once a reagent stock was depleted, it might be very difficult to obtain an identical reagent. From various reagents tested, he assumed a single gene effect determined a pattern of reactions may have been due to antigenic factors controlled by two alleles. Such a pattern would seem to indicate only very gross differences. Yet, such gross differences might be useful in arranging subsequent immunization schedules for producing antisera. Fanguy *et al.* (1961) concluded that "selected reagents produced in inbred populations can be used to identify specific antigens in non-inbred populations through the use of specific cross-reaction patterns." The only real proof given to support

this conclusion was the production of one A, five B, and one or two C reagents when immunizing on the basis of differences in cross-reaction patterns. One would expect to produce antibodies for the A or B systems by injecting between two chickens without any prior knowledge of their blood group antigens.

The same authors state also that "reagents which show single allele specificity in one population may cross react with several alleles of the same blood group system in another population." This is illustrated in Table 23 and is the principal reason why broad spectrum antisera are not reliable in identifying alleles. Thus the usefulness of broad spectrum reagents is limited in studying non-inbred populations and may be of no value in genetic studies that involve identification of alleles.

Reagents that are highly specific in an inbred line may react with many different antigens that have one or more common elements (factors). One such broad spectrum reagent would be almost useless in identifying some specific antigen in a multiple allele system. A number of different reagents would, collectively, distinguish large differences, but would have less value in identifying small differences. If, for example, three B system alleles (B_1, B_2, and B_3) were present in an inbred line and if the antigenic factors for these were: (B_1) 1-2-3-4-5-6-7-8, (B_2) 1-2-3-6-8-10-11-12 and (B_3) 1-2-4-9-10-13-14-15, then the number of theoretical reagents

that could be made for the B system in this line would be as shown in Table 26.

Table 26. Reagents that could be made in an inbred line

Donor	Recipient	Spectrum of antiserum	Reacts with
B_1	B_2	4-5-7	B_1, B_3
B_1	B_3	3-6-7-8	B_1, B_2
B_1	B_2-B_3	5-7	B_1
B_2	B_1	10-11-12	B_2, B_3
B_2	B_3	3-6-8-11-12	B_2, B_1
B_2	B_1-B_3	11-12	B_2
B_3	B_1	9-10-13-14-15	B_2, B_3
B_3	B_2	4-9-13-14-15	B_1, B_3
B_3	B_1-B_2	9-13-14-15	B_3
B_1-B_2	B_2-B_3	5-7	B_1
B_1-B_2	B_1-B_3	11-12	B_2
B_1-B_3	B_1-B_2	9-13-14-15	B_3
B_1-B_3	B_2-B_3	5-7	B_1
B_2-B_3	B_1-B_2	9-13-14-15	B_3
B_2-B_3	B_1-B_3	11-12	B_2

Among the hypothetical reagents made as in Table 26, nine would be specific within this line. Of these, three would result from injecting cells of a homozygote into a bird heterozygous for the other two alleles. This is the basic

method commonly used to make specific reagents in an inbred line. The formation of specific reagents by injecting cells of one heterozygote into a different heterozygote is also possible, as indicated in Table 26, but this method is not commonly used. The non specific reagents may be made specific by adsorbing with cells of the undesired genotype that reacts with the reagent. Thus, a number of reagents can be made specific for the same allele in an inbred line, and perhaps no two reagents would have antibodies against the same antigenic factors. If one were to use the reagents specific for one line in another population with different alleles, then the reagents would no longer be specific, as the alleles in the new population may be composed of different arrangements of the same antigenic factors.

Two reagents with the same specificity in one line may not give identical results in another line. Even though a group of individuals have the same pattern of response to, say, twenty different reagents, some similarity between the various birds might exist, but this could occur without any two birds being identical. This would seem particularly true in multiple allele systems of considerable size such as the B system. In a system with only two alleles, heterologous antisera would be useful. It seems unlikely that a sufficiently large battery of broad spectrum reagents could be obtained to identify alleles in a large multiple allele system such as the B system.

On the other hand, if two birds are found with different patterns of response for the same system, that the two birds are different would seem to be a valid assumption. Injection of cells from one of these birds into the other should lead to formation of antibodies.

Reagent development by adsorbing to the point of maximum specificity is very useful. Even in the very complex B blood group system it was possible with 13 reagents to characterize many alleles in a non-inbred population and through test matings verify the characterization.

The reagents used in this study evolved more or less by chance. The one particular disadvantage of this system is the difficulty in re-duplicating a particular reagent. Reagents could be duplicated by testing a number of birds with specific reagents and eventually finding a few birds where the donor-recipient genotypes were suitable for making a particular reagent. This would be more easily accomplished with highly antigenic systems where the matching of donor and recipient genotypes at a number of loci is not necessary. With some systems where the antigens are not so likely to stimulate antibody response, this would be a difficult process.

Use of Inbred Lines for Reagent Development

The use of inbred lines, particularly where pedigree information is available, offers a number of advantages for the

production of highly specific reagents. Reagents specific within an inbred line can be made rather easily, and additional antisera of approximately the same antibody composition can be prepared as needed. If this antiserum is adsorbed to make one or more highly specific reagents, such reagent stocks could be more easily replaced. In addition, use of inbred lines would make production of specific antisera easier for some less antigenic systems such as C and L.

In this study no C reagents were developed. C reagents are usually weakly antigenic. Development of C reagents usually requires a compatibility between the A, B, D, and E systems of the donor and recipient as antibodies of these systems would obscure C antibodies. This requirement would not often be fulfilled in a non-inbred population. Therefore failure to obtain C reagents in this study is not surprising.

Another advantage of using inbred lines for making specific reagents comes not from advantages in manufacturing antiserum, but in the purification of antiserum. With inbred lines of known genotype, one has available red cells carrying specific antigens that can be used for adsorption to remove the antibodies corresponding to these specific antigens. This is of particular advantage in replenishing a particular reagent where one has some prior knowledge of the reagent desired.

The main disadvantage likely to be encountered in using inbred lines for the production of reagents is the limitation

imposed by lack of antigenic variation within inbred lines. To characterize non-inbred populations, a number of reagents would be needed, particularly for the B system. Development of a large number of different B reagents from one or two inbred lines would not be possible. This disadvantage could be avoided by using a number of diverse inbred lines for reagent production.

Development of Rabbit Reagents

Gilmour (1949) has stated that hetero-immune reagents made in rabbits will not distinguish fine antigenic differences and are of no value in blood typing chickens. In this study, hetero-immune rabbit reagents seemed very useful. Three different reagents were made from rabbit antisera that appeared identical in their specificity for the E2 antigen. No similar reagents were made by iso-immunization, although a very large number of E1 reagents were made. In addition, a number of B reagents were made with rabbit antisera having the same specificity as reagents made by iso-immunization. Antigens for certain blood group systems may be more antigenic in rabbits imposing some limitation as to specificity of reagents that can be developed. However, specific reagents were made from rabbit antisera that appeared to distinguish fine differences among chicken blood group antigens.

Rabbit antisera have certain peculiarities that require

some caution, in their use. Rabbit serum contains complement which must be inactivated by heating at 56°C for 30 minutes, otherwise the red cells being tested may lyse. Inactivated rabbit sera, upon standing, may regain some of its complement activity. If this happens, the antisera may require inactivation again. In settling to the bottom of a tube, cells agglutinated with chicken antiserum usually show an affinity to glass and cover the bottom of the tube. Non-agglutinated cells have no affinity for glass and form a small dot of cells in the center of the tube bottom. Cells agglutinated with rabbit antiserum usually have a sediment pattern resembling the pattern of non-agglutinated cells. As a result, cells agglutinated by chicken antiserum are often easily recognized as agglutinated by an examination of the sediment pattern, even when agglutination is very slight. Agglutination by rabbit antiserum often can be determined only by shaking the tube and examining the cells for clumping. Rabbit antiserum also requires rather careful adsorption to remove species-specific antibodies. Rabbit antiserum may agglutinate all cells after standing several hours, even though it may have given highly discriminating results earlier. This is due to small amounts of species-specific antibodies still present, but can be corrected by proper adsorption. The rabbit antisera used in this study gave excellent results when carefully adsorbed and tested for removal of species-specific antibodies.

Selection Effects on Blood Groups

The results of this experiment indicate that differences in frequency between the blood group alleles of the population tested do exist. More or less rough approximations of the changes that could be expected due to random drift indicate that some force, or forces, other than random drift must have had some effect on the frequencies of the blood group genes. The forces other than random drift that could effect gene frequency are mutation, migration, and selection (Lush, 1948).

Mutations are rare and probably occur with a frequency of about one in 100,000 or 1,000,000 (Gowen and Gay, 1933). In a few generations of a large population such as the one used in this study, the effects of mutation would be negligible and could safely be disregarded as a force for changing gene frequency.

Migration is always a possibility if two or more populations are in close proximity. The birds used in this study were pedigree hatched, banded as removed from the incubators, and then reared and housed together. As a general rule, birds of other breeds and lines were reared separately. The birds were housed in single male pens and hens were trapnested so that parentage of each egg could be determined and marked on the egg. It is unlikely that eggs or birds from other lines could have been mixed in with the eggs or birds of the popula-

tion tested. Errors could occur in marking eggs or a few chicks could be mixed accidentally, but these errors would be within the particular lines in this study. If such errors did occur and a chick was assigned to the wrong line, the chances are that it would be discarded as an adult because it would not reach the selection criterion required for the line in which it was wrongly placed. In any event, such errors of incorrect line classification would reduce rather than increase differences between lines, which statistically is an error of type II. It thus appears reasonable to exclude migration as a cause of differences in gene frequency between the lines in this study.

The remaining force for changing gene frequency is selection. Lines showing differences were under considerable selection pressure and responded by genetic changes in the traits selected. In addition, the lines changed in other traits due to a correlated response to selection. For example, the lines selected for body size showed a large primary response in body size change. Likewise, but to a lesser extent, the lines selected for egg size showed a large primary response and a correlated response in body size.

In this population no intentional selection was made for the blood groups, so that any changes observed in this respect, not accounted for by random drift, would be due to correlated responses to selection. Therefore, those lines that resemble each other most in terms such as egg size and body size should

also resemble each other in frequency of blood group alleles. This is in accord with the findings of this study. The blood group frequencies of the large body-large egg lines were highly correlated and statistically significant as were also the small body-small egg lines. One might also expect that the frequency of the blood groups for the lines selected in opposite directions such as large egg and small egg, would be negatively correlated. In this case, however, they were not negatively correlated, but the correlations were low and not statistically significant.

The reason for the apparent correlated responses of the blood groups with selection for body weight probably involves linkage or pleiotropy. Since body size and egg size are quantitative characters affected by a number of genes that cannot be individually identified, it is unlikely that precise linkage information could be obtained. Any question regarding linkage or pleiotropy as a cause of association of blood group genes with quantitative traits must remain unanswered.

The results of this study demonstrates that blood group genes can be identified and studied in non-inbred populations utilizing the techniques which have been described. This makes possible population studies on blood group gene frequencies and polymorphisms.

Some refinements might possibly improve the experimental design for maximum use of the blood group information. For

example, four way crosses of inbred lines with known blood group alleles would yield a synthetic population with a known number and frequency of specified blood group alleles. This might be advantageous in further studies of the effects of selection and random drift. Also random drift could be more accurately assessed by replication of lines.

SUMMARY

The possible association of blood groups with economically important traits is of interest to animal and poultry geneticists. Such association would permit utilization of qualitative blood group information as an indicator of the genetic material controlling quantitative traits. Blood typing may also be a useful aid in breeding programs in the detection and elimination of individuals with pedigree errors. In addition to the practical uses of blood typing techniques, utilization of blood group information for more basic genetic studies on populations is of importance.

Blood typing reagents in chickens are usually produced by immunizing an individual with red cells from another individual of the same inbred line. Reagents produced by this method are often satisfactory for identifying an antigenic complex controlled by a single allele within the same inbred line yet such are usually unsatisfactory when used in a non-inbred line because of the phenomenon of "cross reaction". That is, reactions may occur with various antigens having common elements but controlled by different alleles of the same blood group system.

The purpose of this study was to develop specific reagents suitable for use in a non-inbred population, demonstrate the heritable nature of the antigens reacting with these reagents, and utilize these reagents to characterize a non-inbred population. The characterized blood groups were studied to determine whether selection for quantitative traits had influenced blood

group gene frequencies.

The population studied was the fifth generation of five lines selected for single quantitative traits as follows: A line, egg production; B line, large body size; C line, small body size; D line, large egg size; and E line, small egg size. These lines originated from a base population produced by four way crosses of four commercial strains of White Leghorns. Selection for egg size or body size not only changed the trait selected, but also changed the other trait due to a correlated response to selection. Thus the large body-large egg lines and the small body-small egg lines showed similar responses to selection.

Reagents were developed by injecting chicken red cells into rabbits (hetero-immunization) or chickens (iso-immunization) and harvesting the resulting antisera. The antisera were repeatedly adsorbed with red cells and tested by tube agglutination tests in attempts to make reagents specific for the smallest possible part (antigenic factor) of a blood group antigen. This corresponded approximately to the procedures commonly used in developing reagents for cattle blood typing. Reagent development required more than 3,000 adsorptions and 120,000 tube tests.

In all, 96 reagents of rabbit source and 181 of chicken source were developed. Ninety-two selected reagents were used in characterizing inbred lines of known genotypes indicated these reagents to be specific for one allele in each of the A, D, X, and Z systems, two alleles in the E system, and 13 different antigenic factors of the B system. In addition, eight reagents ap-

peared specific in reactivity, but could not be assigned to a blood group system.

Birds chosen as breeders in 1962 were tested with 92 selected reagents. In addition, parents and progeny of some of these birds were tested. In all, more than 110,000 tube agglutination tests were made in characterizing the population.

For some families, three generations were tested in order to study the genetic segregation of the identified B system antigens. The B system reagents identified antigenic factors present in a number of different combinations. Each different combination was assumed to be controlled by a different allele. Genotypes were postulated for a parental generation based on these combinations. Only those phenotypes that were predicted were found in the progeny, indicating that where sufficient reagents were available, the characterized B antigens were allelic.

The reagents for the systems other than B identified antigens that apparently segregated in the genetic ratios expected for single loci. In addition, the antigens identified by the eight reagents of unknown blood group systems appeared to segregate as independent single loci.

The frequencies of the blood group alleles for all identified and unidentified systems, except B, were estimated. Differences in gene frequency between lines for a single allele varied as much as .48. The possible effects of random drift were also considered. The estimated gene frequencies of eleven alleles differed more than that expected from random drift alone. Dif-

ferences not due to random drift were deduced to be the result of selection for quantitative traits. Gene frequencies of those lines similar in body weight and egg size were more highly correlated (large body vs. large egg lines, .92 and small body vs. small egg lines, .94) than those lines that were different (large body vs. small body lines, .84 and large egg vs. small egg lines .76).

The A line seemed not to have changed appreciably due to selection for egg production. The partial correlations between the lines holding the A line constant, showed large body vs. large egg lines and small body vs. small egg lines to have statistically significant correlations of .92 and .73, respectively. All other correlations were low and positive, but not statistically significant. These results indicate that changes in blood group frequencies are correlated responses to selection for egg size and body size.

The methods used in this study contrast to a technique used in attempts to characterize non-inbred populations by patterns of reaction to locus specific reagents containing a broad spectrum of antibodies. The results obtained in this study probably could not have been obtained using such a technique, and based on theoretical considerations, broad spectrum reagents were not believed adequate for genetic studies.

The highly specific adsorbed reagents developed and used in this study gave consistent and reliable results. The method

of characterizing blood group alleles of non-inbred population using specific reagents was precise and accurate. Hence, the potential value of this method was clearly demonstrated.

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APPENDIX A

Table 27. Blood group characterizations of the population tested

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
G8811	1	6-9-14	-	-	-	1	69-170
G6226	-	6-9	-	1-2	1	1	145
G8238	1	3[a]-5-6-7-12-13	-	1	1	1	141-145
G8919	-	7-12	-	-	-	1	69
G10981	1	3-5-6-7-9-11-13	-	1	-	1	69-145-170
G11245	-	4-7-9-10-12-14	-	2	1	1	82-133-141-142
G11017	1	3-5-6-7-9-12-13-14	-	2	-	1	141
G11426	-	6-9	-	2	1	1	69-141
G8100	1	7-9-10-12	-	1-2	1	1	69-170
G6051	1	7-12	-	1	1	1	82-141-142-145
G8243	-	7-9-12	-	2	1	1	82-141-142
G8839	-	6-9-12-14	-	-	-	1	141-170
G11532	1	5-7-13	-	1	-	1	170
G11441	-	5-7-9-10-13	-	-	1	1	69-82-133-141-142
G8275	1	3-5-7-9-13	-	1	1	1	69-145-170
G8797	-	9	-	-	-	1	69
G8806	1	7-9-12	-	-	-	1	69
G11286	-	7-9-12	-	-	-	1	69
G11791	-	7-9-10	-	-	-	1	82-141-142-170
G11481	-	7-11-12	-	-	1	-	69
G8178	1	4-7-12	-	1	1	1	69-145-171
G11883	-	6-12	-	2	-	-	69-141-170
G8155	1	4-12	1-	2	1	-	142-141
G8673	1	2-3-4-7-12-13	1	2	1	-	69-170
G11222	1	4-7-9-12-13	-	1	1	1	69-145
G8999	1	4-7-12	-	1	-	1	69-145-170
G8942	1	4-7-10-11-12	-	2	1	1	69-82-142-141-170
G11019	1	2-3-7-9-12-13	-	2	-	1	69-170
G11605	-	4-6-7-12	-	-	1	-	141
G11549	1	4-7-10-12	1	1	1	-	141
G11452	1		-	-	1	-	69
G6039	-	10-11-14	-	1-2	1	-	145-141
G8821	1	1-2-3-12-13	-	-	-	-	69
G6012	-	4-7-12	-	1-2	1	1	69
G11308	1	2-4-12-14	-	2	1	-	69-82-169-170

[a]Underlining indicates questionable readings.

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
G8216	1	4	-	-	1	1	82-142
G11606	-	4-12-14	-	2	1	-	69
G8763	1	4-10-12-13	-	1	1	1	69-142
G10854	1	1-2-3-10-12-13	-	-	1	1	69
G8829	1	1-2-3-10-12-13	-	-	1	1	69
G8829	1	1-2-3-4-13	-	2	1	-	69
G6014	1	2-3-4-13-14	-	1-2	1	1	69-82-170
G11224	-	4	-	2	1	-	69
G6177	1	6-9-12	-	1	1	1	69-145
G6075	1	9	-	1	1	1	69-145
G8630	1	$\overline{4}$-6-9-14	-	-	1	1	69
G8215	1	2-3-10-11-13	-	-	1	1	141
G8813	1	$\overline{6}$-$\overline{9}$-12	-	-	1	-	69
G11612	1	4-9-14	-	-	-	-	69
G11818	1	4-$\overline{1}$4	-	1	-	1	69-171
G10839	1	6-10-11-12-14	1	1	1	1	141-142-169-171
G8743	1	4-10	1	1-2	1	-	69-141-142-145-169-171
G8644	-	10-11-13	-	2	1	-	141-142
G8472	-	4-9-14	-	2	1	-	69
G11636	-	4-9	1	2	1	-	69
G11619	1	6-9-12-14	-	1	1	1	69-145-171
G10848	1	9-10-14	-	-	1	-	69
G8945	1	$\overline{4}$-6-7-9-k2-14	-	-	-	1	69-170-172
G11443	-	6-9-14	-	2	1	-	172
G8306	1	2-7-13	-	-	1	-	172
G8245	1	$\overline{2}$-3-4-7-13	-	-	1	-	172
G11250	1	$\overline{4}$-7	-	1	1	1	145-171
G11614	1	2-3-6-12-13-14	-	-	-	1	
G11638	-	$\overline{2}$-3-6-7-12-13	-	-	-	-	172
G10880	1	$\overline{4}$-7-10	-	1	1	1	142-171
G8791	1	7-9	-	-	1	1	69
H7267	1	4-6-7-9-12-14	-	2	1	1	69
H3316	1	6-9	-	1	1	1	69
H3520	1	3-4-6-7-9-12-13-14	-	2	1	1	64
H3298	1	$\overline{6}$-9-10-11-12	-	1-2	1	1	69-141-82
H3243	1	4-6-7-11-14	-	2	1	-	82
H7331	1	2-4-6-7-12-13-14	-	1	1	1	
H3268	1	2-3-4-6-7-12-13	-	1	1	1	
H7220	1	2-4-6-7-12-13-14	-	-	1	1	
H7234	1	4-7-12-13-14	-	-	1	1	27-69-142
H7376	1	7-9-11-12-13-14	-	2	1	1	82-141-142
H3048	1	7-9-11-12-13-14	-	1-2	1	1	82-141-142

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H7193	1	6-9	-	1-2	-	1	64-170
H3074	1	9-12	-	1	1	1	69
H3318	1	6-9	-	1	-	1	69
H3519	1	6-9	-	2	1	1	69
H3297	1	3-6-9-11	-	1-2	1	1	142-82-133-145
H4951	1	4-7-12-13-14	-	-	1	1	
H7429	1	2-3-4-6-7-13	-	1	1	1	145
H7452	1	6-7-12-13	-	1	1	1	
H7332	1	4-6-7-9-11-12-13-14	-	1	1	1	
H7278	-	4-7-10-11-13-14	-	2	-	1	82
H7377	1	4-7-10-11-13-14	-	1-2	1	1	82-142-133
H4927	1	4-6-7-12-14	-	2	1	1	
H3227	1	2-6-6-13	-	1-2	-	-	69
H3317	1	6-11-12-14	-	1	1	1	120
H3062	1	2-6-7-12-13	-	1-2	1	1	69
H3525	1	6-9-14	-	-	1	1	
H5150	1	2-6-9-13	-	1	1	1	69
H7264	1	6-9	-	1-2	1	1	
H7430	1	2-4-6-7-13-14	-	2	1	1	69-170
H7346	1	6-7-12	-	1-2	1	-	
H7618	1	4-7-11	-	1	1	-	142
H7449	-	2-6-7-13-14	-	2	1	1	
H3114	1	7-11-14	-	2	1	1	170
H3464	1	6-7-9-14	-	2	1	-	69
H3311	1	2-6-7-9-13	-	1-2	1	-	66
H3248	1	4-6-7-14	-	1	1	1	
H3235	1	4-7-13-14	-	1-2	-	-	82-142
H3406	1	4-6-7-9-12-13-14	-	2	1	1	69
H3066	1	4-7-11-13-14	-	1-2	1	1	133
H5375	1	6-9-14	-	2	1	1	69
H7480	1	6-7-12-13-14	-	1-2	1	1	69
H7346	1	4-6-7-12-14	-	1-2	1	-	
H7572	1	2-3-10-13-14	-	1	1	1	145
H3398	1	9-10	1	1	1	-	69-141-142-145
H3516	1	4-7-12-14	-	2	-	1	69-170
H3200	1	7-10-11	-	1	1	1	141-142-145
H3414	1	7-10	-	-	1	-	142
H3465	1	7-9	-	-	-	-	69
H3252	-	4-7-12-14	-	2	-	-	
H3064	1	4-7-10-11	-	2	-	1	82-141-142
H5348	1	4-7-9-12-14	-	-	1	1	69

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H4928	1	6-7-12	-	-	1	1	142
H7262	1	4-9-11	-	1	-	1	69-141-133-142-169
H7453	1	2-7-13	-	1	1	1	69-145
H3467	-	4-6-14	-	2	1	-	69
H3393	1	7-9-10-11-14	-	1-2	1	-	82-141-145
H7483	1	4-7-12	-	-	1	1	69-145
H3181	1	7-9	-	1-2	1	-	69-145-169
H3517	1	4-7-12-14	-	2	-	1	69
H4916	1	4-7-10	-	-	1	-	141
H5163	1	4-6-10-12	-	1	1	-	141-145
H7719	1	4-7-14	-	1	1	1	
H7268	1	6-7-12	-	1	0	1	
H7212	1	7	-	1	1	1	
H5046	1	6-7-12-14	-	1-2	1	-	
H3477	-	6-7-9-14	-	2	1	1	69
H3085	1	6-7-9	-	-	1	1	69
H3058	-	6-7-9-13	-	2	1	-	69
H3179	1	4-6-7-12	-	1-2	1	-	
H3249	-	6-7-12-13	-	2	1	-	
H3445	1	6-7-12-13	-	2	-	-	
H5395	1	9-14	-	-	1	1	
H7523	1	6-9-11-13	-	2	1	-	133
H7729	-	6-7-9	-	1-2	1	-	69
H3250	-	4-6-12-14	-	2	1	-	169-170
H5503	1	6-9-10-14	-	1-2	1	1	
H3061	1	6-9-14	-	1-2	1	1	69-169-170-171
H3087	1	6-9	-	2	1	1	69
H3397	1	6-9-10	-	1	1	1	145-170-171
H5295	1	6-12-14	-	2	1	-	69-169
H4934	1	7-12-14	-	-	1	1	69
H7524	1	6-9-10-11	-	1	1	1	133-145
H7551	1	7-12	-	1-2	1	1	69-170-171
H7725	1	4-7-12-14	-	-	1	1	69-169-170
H5170	1	6-9-11	1	1-2	1	1	69-170-141-142-133-145-169-171
H3082	1	6-7-11	-	1	1	1	133-170-171
H3280	1	4-7-12-13-14	-	2	1	-	170
H3084	1	6-7	-	2	1	1	142-170
H5355	1	6-9-14	-	-	1	1	69
H5077	1	6-7-12	-	-	1	-	
H7192	1	1-2-3-6-7-13	0	1	1	1	170-171

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H7709	1	6-7-9	-	1-2	1	1	170-171
H7350	1	6-7	-	1-2	1	1	
H7656	1	2-3-6-7-9-13-14	-	2	1	-	170
H7517	1	6-9-11	-	1-2	1	-	69-82-142-133-171
H3386	1	6-7-14	-	2	1	1	69-170
H3109	1	6-7-9	-	2	1	1	69-170
H3353	1	7-13	-	1-2	1	1	170-171
H5358	1	7-12-13-14	-	-	1	-	69
H4930	1	6-7-9-12	-	-	1	1	69
H7421	-	7	-	2	1	-	
H7195	1	4-7-13	-	1-2	1	-	69-171
H7223	1	7-11-14	-	2	1	1	171
H7655	1	2-6-9-13	-	1	1	-	
H3080	1	4-7-10-11-14	-	-	1	-	69
H3056	-	7-14	-	2	1	1	69-170
H3388	1	4-7-12-14	-	1	1	1	69-145-170-171
H3119	1	4-7	-	1	1	1	
H3111	1	6-9-10-11	-	-	1	-	133
H5050	1	6-7-12-14	-	2	1	-	
H5120	1	4-6-7-12-14	-	2	1	-	69
H7722	1	4-7-14	-	2	1	-	170
H7422	1	6-7-11-12-14	-	-	1	-	
H7521	1	6-9-10-11	-	1	1	-	133
H3396	1	7-10-14	-	1-2	1	-	145-170-171
H3113	1	6-7-9-14	-	-	1	1	69-171
H3394	1	6-9-10-14	1	1-2	1	-	170-171
H3209	1	6-7-13	-	1	1	1	
H3454	1	6-7-9-14	-	2	1	-	69
H3117	1	4-7	-	-	1	-	
H4931	1	6-7-9-12	-	-	1	-	69
H7724	1	7-12-14	-	-	1	-	171
H7226	1	6-7-12	-	1	1	1	
H7423	1	6-7-12-14	-	-	1	-	
H7733	1	6-9-12-14	-	-	1	1	69-169-170
H3267	-	6-7-9-12-14	-	-	1	-	
H3050	1	4-7-11	-	1	1	-	27
H3007	1	1-2-3-6-9-11-12	-	2	1	-	27-133-169
H3212	1	3-4-6-7-14	-	-	1	-	69
H3355	1	4-6-7-12-14	-	-	1	1	170
H3319	1	6-9-12	-	1	1	-	69-169
H3118	1	7	-	1	1	-	
H3094	1	4-6-7-9-12	-	1	1	1	69-145

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H7227	1	4-7-11-14	-	-	1	-	142-169
H5296	-	6-9-12-14	-	2	1	-	169-170
H3266	-	6-7	-	-	1	-	
H3112	1	4-7-14	-	-	1	1	27
H3049	1	4-6-7-9-12	-	1-2	1	1	27-69-145-170-171
H3120	1	4-7	-	1	1	-	145
H3079	1	4-7-14	-	-	1	1	142-133
H4907	1	4-7-14	-	1-2	1	1	69-82-171
H7721	1	6-9-12-14	-	-	1	1	27-69-170
H7384	1	1-2-3-4-6-7-14	-	-	1	1	170
H7533	1	4-6-9-12	1	1	1	1	142-133-145-170
H7241	1	4-6-7-9-12-14	-	2	1	1	170
H7734	1	6-9-14	-	-	1	1	169-170-69
H3163	1	7-11	-	1	1	-	
H3270	1	6-7-12	-	1	1	1	69
H3244	-	7-11-12	-	-	1	-	69
H3093	1	4-6-7-14	-	-	1	-	
H3205	1	1-2-3-5-7-14	-	-	1	-	
H3305	1	6-9-14	-	1	1	-	
H4952	1	4-7-12	-	1	1	-	145-69
H4953	-	7-11-12-14	-	-	1	-	
H7187	1	6-7-9-11	-	1-2	1	1	69-133
H7161	1	6-9-14	-	-	1	1	69
H7728	1	4-7-10-14	-	-	1	-	
H3402	1	4-7-11-14	-	2	1	-	69
H3246	1	4-7-11	-	1	1	1	
H3165	1	4-7-11	1	1	1	1	133
H3304	1	6-9-14	-	1-2	1	-	145
H3269	1	6-7-12	-	1	1	-	145-169
H7275	1	6-10	-	2	1	-	69-133-169
H7232	1	1-2-3-4-5-6-7	-	-	1	1	
H7188	1	4-7-11	-	1-2	1	1	69
H7433	-	4-7-11-14	-	2	1	-	69
H7379	1	6-7-9	-	1	1	-	
H7822	1	4-7-12-13	-	2	1	-	82-69-142-170
H3596	1	6-12-14	1	2	-	1	82-141
H3574	1	6-7-12-14	1	-	-	-	141-142
H3578	1	4-6-7-12-13-14	-	2	1	1	69
H3626	-	4-6-7-12-13-14	-	1-2	1	1	69
H5622	1	4-6-9-12-14	-	2	1	1	27-69-141
H5634	-	4-7-12-14	1	2	1	-	69
H7899	1	4-7-12	-	1	-	-	27-69

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H10414	-	4-6-7-9-12-13-14	-	1-2	1	1	82-170
H8068	1	4-7-11-12-13-14	-	2	1	1	82-141-142
H7910	1	4-7-14	-	-	-	-	142-170
H7834	1	3-4-6-7-12-13-14	1	1-2	-	1	82-69-170
H5639	1	4-6-7-9-12-13-14	-	2	1	-	69
H5722	-	2-3-4-6-7-9-12-13-14	-	1-2	1	1	69-170
H5651	-	4-6-7-9-12-13-14	-	2	-	1	69-170
H8064	-	4-6-7-9-12-13-14	-	2	1	1	69-170
H7878	1	6-10-11-12	1	1	1	-	82-69-142-133
H8024	1	4-7-12-13-14	-	2	1	1	27-170
H7897	1	7-11	-	1-2	1	-	82-142
H7912	1	7-12	-	1-2	-	-	170
H10295	1	7-12-14	-	2	-	1	69-170
H10340	1	7-11-12-14	-	2	1	-	142
H7906	1	7-10-11	-	-	1	-	141-142
H3559	-	7	-	1-2	1	-	69-141-142-145
H3597	1	7-9-14	1	2	-	1	69
H5623	-	7-10-11-14	-	1-2	1	1	69-145
H5563	1	6-7	-	1-2	1	-	69-171
H5640	-	2-3-5-6-7-9	-	2	-	1	69
H8028	1	6-7-12-14	-	2	-	1	169-170
H8062	-	4-7-9-14	-	1-2	1	-	69-145
H8057	-	2-4-5-7-13-14	-	2	1	-	
H7891	1	6-7-12	-	1	1	1	
H10344	1	4-7-10	-	1	1	1	141-142-169-170
H3617	-	6-9-12-14	-	1	1	1	145-169-170
H3556	1	6-9-14	-	2	1	-	69
H5702	-	2-3-6-9-13-14	1	2	1	1	69
H5584	1	7	-	-	-	-	142
H5530	1	7-14	-	1	1	-	
H5624	1	4-7-10-11-13-14	1	2	1	1	82-141-142-133-169-170
H7967	1	2-4-7-10-11-13-14	-	2	1	1	141-142-133-169-170
H7936	-	4-7-9-13	-	2	-	1	69-170
H7892	-	2-3-6-7-11-12-13-14	-	1-2	1	-	69-170
H10384	1	4-7	-	1	1	1	69-170
H8018	1	4-7-13-14	-	2	1	1	
H3605	-	4-6-7-9-14	1	1-2	1	1	69-145
H3582	-	7	-	-	1	-	

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H3603	1	4-6-9-12-14	1	1-2	1	1	69-169-170
H3623	-	9	1	1	1	-	69-145
H5549	1	6-7-12-14	-	1	1	1	169-170
H8052	-	4-7-12	1	1	1	1	145
H7938	-	4-7-9-13-14	-	2	1	1	69
H7951	-	7-9-13-14	-	1-2	1	-	68-145
H10379	1	1-4-7-13	1	2	1	1	69
H7813	-	9	-	1	1	-	69-145
H3599	-	6-9-12-14	-	1-2	1	1	69-145-169
H3584	1	7	-	1	1	-	
H3598	1	6-7-10-11-12-14	-	2	1	1	141-142-133-169
H3552	1	4-7-14	-	2	1	1	69
H5513	1	9-	-	-	1	-	69
H5547	1	6-7-12-14	-	1	1	1	169-170
H7865	1	6-7-12-14	-	1	1	1	
H7940	-	4-7-12-13-14	1	2	1	-	
H10388	1	6-9-12-14	-	2	1	1	169
H7943	1	2-3-6-7-12-13-14	-	1-2	1	1	
H7816	1	4-7	-	-	-	-	
H8009	-	6-9-12	-	1-2	1	1	169
H3604	1	9-14	-	1-2	1	1	69
H3557	1	4-7-10-13	-	-	1	1	82-141-142-133-169
H5617	-	9-10-14	1	1-2	1	1	69-141-142-133-
H5544	1	6-7-12-14	-	-	1	1	169 145-169
H7925	1	9-13-14	1	2	-	1	69
H7928	1	7-12	-	-	1	1	69
H7965	1	6-7-12-14	-	2	-	1	169
H7851	1	-	-	1	-	-	
H10341	-	7	-	-	-	-	
H8056	-	2-3-4-7-12-13-14	-	2	1	1	170
H7991	1	4-7-9-13-14	1	2	1	1	69
H3571	1	6-7-12-14	-	1	-	1	169
H3583	1	7-13	-	-	-	-	145
H5502	1	2-3-5-7-13-14	-	-	1	1	69
H5518	-	4-7-10-13-14	1	1-2	1	-	141-142-133-145-169-170
H5583	-	7	-	-	1	-	142
H5660	1	7	-	-	1	1	145
H5575	1	7-10	-	-	1	-	141-142-133-169-170
H10367	1	6-10-12-14	1	2	-	1	141-142-169

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H7923	-	6-7-9	-	1-2	1	-	69-145
H8061	6-7-9-13		-	1-2	1	-	145
H5809	1	4-6-7-9-13-14	-	2	1	-	82-141-142-170
H3937	-	2-3-4-6-7-13-14	-	2	1	1	
H3919	-	6-9-11-12-14	-	2	1	-	141
H3829	1	2-3-5-6-7-13-14	-	-	1	-	
H3946	-	6-9-11-12	-	2	1	-	141
H5944	1	2-3-5-6-7-12-13-14	-	2	1	1	145
H8233	1	4-6-7-9-12-13-14	-	2	1	1	69-27-142-145
H10605	-	6-7	-	-	1	-	69-27
H10485	1	2-3-4-5-6-7-13	-	-	1	1	
H8245	1	6-9-12-13-14	-	-	1	1	69-141
H8252	1	3-4-5-6-7-9-11-12-13	-	2	1	1	69-82-141-142-145
H3982	1	4-6-9-12-13	-	2	1	1	170
H3785	1-2-3-6-7-13-14		-	2	1	-	27-171
H3956	-	4-5-6-12-14	1	2	1	-	142-171
H3831	1	2-3-4-6-7-13-14	-	2	1	-	27-69-171
H10352	1	4-6-7-9-12-13-14	-	2	1	-	69
H10514	1	2-3-6-13	-	2	1	-	
H10589	-	6-12	-	2	1	-	
H8340	1	2-6-7-13	-	-	1	-	
H8180	-	6-12-14	1	-	1	-	69
H8355	1	4-7-12-13-14	-	2	1	-	27
H8358	-	6-9-12-14	-	2	1	-	
H3816	1	2-3-4-7-13	-	2	1	-	
H3948	-	6-7-12	-	-	1	-	142
H3883	-	7-9	-	2	-	1	69
H3923	-	2-3-6-12-13	-	-	1	-	
H3868	1	2-3-6-7-12-13	-	-	1	-	
H3698	1	7-12	-	2	-	-	169-170-69
H10476	-	6-12	1	2	-	-	
H10551	1	2-3-9-13	-	-	1	1	
H10540	1	2-3-6-7-12-13	-	-	-	1	170
H8352	-	6-7-11-12	-	-	-	-	
H8379	-	2-3-6-7-12-13	-	-	1	-	
H5940	1	2-3-6-12-13-14	-	2	1	1	170
H3732	1	2-3-4-7-13	-	2	1	1	69-170
H3842	1	2-3-6-12-13-14	-	-	-	1	170-165
H3926	-	2-3-6-12	-	2	1	-	169
H3685	1	2-3-5-6-13	-	-	1	-	169

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H3832	1	2-3-5-6-12-13	-	-	1	1	169
H5054	-	6-12	1	-	1	1	69
H6102	1	6	-	2	1	1	
H6021	1	2-3-7-13	-	-	1	1	
H10597	-	4-7-13	-	-	1	-	
H8359	1	3-6-12	-	-	1	-	
H5995	1	1-2-3-5-6-7-13	-	2	1	-	69-169-170
H3887	-	12	-	-	1	-	
H3941	-	6-12	-	-	1	-	169
H3808	1	1-2-3-6-7-9-13	-	2	1	-	69
H3797	1	6-9-12	-	-	1	-	69
H3733	1	1-2-3-4-7-12	-	-	1	1	69
H3818	1	1-2-3-4-7-12-13	-	-	1	1	69
H3766	1	6-9-12	-	-	1	1	69
H5952	1	1-2-3-6-12	-	-	1	-	
H6061	-	6-7-12	-	2	1	-	69
H10525	1	1-2-3-9-13	-	-	1	1	69
H6027	1	2-3-4-13	-	-	1	1	
H3931	-	2-3-6-12-13	-	-	-	-	
H3847	1	2-3-6-12-13	-	2	1	1	
H3740	1	6-12	-	-	1	1	
H3944	-	6-12	-	2	1	-	
H5965	1	2-3-6-12-13	-	2	1	1	
H5980	1	4-9-13	-	2	1	1	69
H5939	1	6-9-12	-	-	1	1	69
H8228	1	6-9	-	-	1	1	69-133
H8158	1	2-3-9-13	1	-	1	1	133-145
H3954	-	6-7-12-14	-	2	1	-	
H3819	1	3-6-9-12-13	-	1	1	1	69-145
H3801	1	2-3-6-7-12-13	-	-	1	1	
H6010	1	6-7-12	-	2	1	-	170
H6029	1	2-3-6-12	-	2	1	-	
H8190	1	7-13	-	1-2	1	-	69
H10565	-	3-7	-	-	1	-	
H10467	-	7-13	-	2	1	1	
H8248	1	2-3-7-13	-	2	1	1	69
H8216	1	6-9-12-14	-	-	1	1	69-169
H8231	1	1-2-3-5-6-7-12-13	-	-	1	1	169
H8367	-	6-7-12-14	-	2	1	-	
H3885	-	1-2-3-5-6-12-13-14	-	2	1	1	170
H3970	1	6-12-14	-	2	1	1	170
H3856	1	6-7-9	-	-	1	-	69

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H3973	1	6-12	-	2	1	1	69
H3741	1	1-6-7-12-14	-	2	1	1	69-145
H8250	1	4-6-7-9	-	2	1	1	69
H8241	1	6-9-12-14	-	-	1	1	69
H8219	1	2-3-5-6-7-14	-	-	1	1	
H10475	1	2-3-6-7	1	2	1	-	69
H10568	-	7-12	-	-	-	-	
H8430	-	4-7-12-13-14	1	2	1	-	170
H4134	1	4-6-7-12	1	1-2	1	1	169
H4094	1	2-3-4-6-7-13-14	1	2	1	1	
H4120	1	6-9-11-12-14	1	1-2	1	1	82-133
H6195	1	6-12-14	-	-	1	1	
H6278	1	9-11	-	1-2	1	1	82-133
H6354	-	6-7-12	1	2	1	-	
H10893	1	6-9-11-14	1	2	1	1	82-133-169
H10698	1	2-6-7-12-13	-	-	1	1	169
H10890	-	6-9-11-14	1	2	1	-	82-133-169
H8526	1	6-9-11-12	-	1	1	1	82-133-169
H10705	1	6-9-11-14	-	2	1	1	142
H4137	-	2-3-5-6-9-11-13-14	1	2	1	-	142
H6383	1	4-7-11	1	1-2	-	-	142-133-170
H6417	-	9-14	1	2	1	-	142-133-169
H8771	-	2-3-5-6-7-11-13	1	2	1	-	142
H8777	1	4-7-11-12	1	-	1	-	69-142-133
H10879	-	4-7-11-12	1	-	1	-	142
H10791	1	6-9-11-14	-	1-2	1	1	69-142-145
H8673	1	2-3-6-7-13-14	1	2	-	-	69
H8527	1	3-6-9-11-12-14	1	1-2	1	1	27-69-141-133-145-169-171
H8402	1	2-3-6-7-13-14	1	2	-	1	69
H4015	1	6-12	1	1	1	1	
H4151	1	6-11-12-14	1	1	1	-	
H4114	1	2-5-10-11-13	1	1	1	1	141-142
H6231	-	6-12-14	1	1	1	-	
H6482	-	2-3-5-6-7-11-12-13-14	1	-	1	-	
H8745	-	7-10-11-14	1	1-2	1	-	69-141-142-170
H8414	1	2-3-5-5-6-7-10-11-12-13-14	1	1-2	1	1	27-69-82-141-171
H10881	-	6-9-11-12	1	1-2	1	1	170
H10793	1	7-10-11	-	-	1	-	141-142
H8674	1	2-3-7-13	1	-	1	1	170
H4129	-	4-7-10-11-14	1	2	1	-	141-142-169-170
H4100	1	2-3-4-6-7-14	1	-	1	1	69-170

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H4083	1	6-7-12	1	-	1	1	170-169
H10781	1	2-3-6-7-9-11-12-13	-	1-2	-	1	145-170-169-171
H10803	-	6-10	-	-	1	-	141-142
H8478	-	6-12-12	-	1-2	1	-	69-141-142-169
H8583	1	4-6-7-12-14	1	1-2	1	1	142-145-169-171
H8537	1	6-12-14	1	1-2	1	1	27
H8418	1	1-2-3-6-7-9-13	1	1-2	1	1	
H8491	1	6-11-12-14	1	1-2	-	1	145-169
H8722	1	1-2-3-4-6-7	-	-	1	1	
H8601	-	4-7-10-11-14	-	2	1	1	68-142-170
H4801	-	7-10	1	2	1	-	142
H4022	1	11-12	-	1	1	-	142
H4089	1	4-6-14	1	-	1	1	69
H4008	1	6-11-12-14	-	1	1	-	69-145-169
H4126	-	6-12	1	2	1	1	69
H6174	1	2-3-7-11-12-13	-	1	1	1	69-145-170-171
H6240	1	6-10-11-12	-	1	1	1	69-141-142-170-171
H8759	-	6-7-11	1	-	1	-	141-142
H8595	1	6-10-11-12	1	2	1	-	69-141-142
H8419	1	6-12	1	1-2	1	-	69
H4095	1	1-2-3-4-12-13	1	1	1	1	170
H4131	-	6-12	1	2	1	1	169
H6175	1	2-3-9-12	-	1	1	1	
H6189	-	6-12	-	1-2	1	-	169
H6205	1	2-3-6-12	-	1	1	-	
H8690	1	7-9-12	-	1	-	1	171
H8760	-	12-14	1	2	1	-	69
H10866	1	2-3-9-12	-	2	1	1	69
H10755	1	6-12	-	1	1	1	69-169
H8629	1	6-7-12	-	1	1	1	69-133
H8431	1	7	-	2	1	1	170
H4130	-	6-11-12	1	2	1	1	169-170
H6177	1	6-7-12-14	-	1-2	1	1	169-171
H6298	-	12-14	-	1	1	1	133
H6248	1	9-14	-	1-2	1	-	27-69-142-133-170-171
H6262	1	9-11	-	-	1	1	169
H8479	1	6-11-12	-	-	1	1	169
H8734	1	2-3-12-14	1	-	1	1	170
H8505	-	7-9-12	-	1	1	1	

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H8725	-	6-9-12	1	-	1	1	69
H8436	1	9-12	1	2	1	-	69
H8659	-	4-12	1	1	1	1	171
H6405	-	2-3-7-12-13	1	2	1	-	169
H4014	1	12	-	1-2	1	1	169-171
H4092	1	2-3-6-7-12	1	2	1	1	
H6271	1	3-9-12-14	-	1-2	-	1	27-69-169-171
H10696	1	6-12	-	-	1	-	
H8729	1	7-9	1	2	1	1	69
H10672	1	2-3-9	-	2	1	-	69
H10853	1	2-3-9-13	-	1-2	1-	1	69
H8630	-	10-11-12-14	-	1-2	1	1	141-142-169
H8506	1	6-11-12-14	1	1-2	1	-	169
H6581	1	4-6-7-13	-	1-2	1	1	142-170-171
H4392	1	4-6-7	-	2	1	-	
H4421	1	4-6-7	-	1	1	1	142-69
H4381	1	6-14	-	-	1	1	141-142-69
H4320	1	4-6-7	-	2	1	1	82-141-142-69
H4450	1	6-7	-	1	1	-	69
H6785	1	6-9	1	1	1	1	142-170-69-171
H6616	1	4-6-7-14	-	2	1	1	142-69
H9147	1	4-6-7	-	1	1	1	82
H9078	1	2-3-6-7-13-14	-	2	1	-	27
H8915	1	4-6-7-9	-	1-2	1	1	142-170-69
H6570	1	6-7-11-14	-	1	1	1	82-170
H4468	1	4-6-7	-	1	1	1	82
H4393	1	6-7-12	-	-	1	-	82
H4332	-	4-7	-	2	1	-	69-82
H6612	1	4-6-7	-	2	1	1	
H6658	1	6-9	-	1	1	1	69-170
H9151	1	4-6-7	-	1-2	1	1	
H9079	1	1-2-6	-	2	1	-	69
H9139	1	4-6-7-12	-	2	1	-	
H9137	1	4-6-7-12-14	-	1	1	1	69
H4297	1	4-7-14	1	-	1	1	69
H4257	1	4-7	1	-	1	1	69
H4229	1	1-2-3-4-6-7-9-12-13-14	-	1-2	1	1	27-69-82-133-171
H4265	1	6-7	-	-	1	1	64-142
H4362	1	7	-	-	-	-	
H6686	1	4-7	-	-	-	1	69
H6713	1	6-7-9	1	-	-	-	69
H6748	1	7	1	1	-	1	69-145-171

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H6641	1	6-7-9	-	-	1	-	69
H9183	1	6-7	1	-	1	-	69
H8976	1	6-7-11-12	-	1	-	1	69-142-145-169-170-171
H4426	1	7-12	-	2	1	-	169
H4237	1	9-11-14	-	2	1	-	69-169-170
H8709	1	7-9-12-14	-	1	1	1	27-69-170-171
H4378	1	2-3-7-14	-	2	1	-	69-145-169-170
H4363	1	7-12	-	-	1	-	170
H6505	1	7-12	-	1	1	1	145-170-171
H6790	1	4-7-10-12	-	-	1	1	142
H6714	1	4-9	1	-	1	-	69
H9038	1	9	-	1	-	1	27-69-145-169-170-171
H4366	1	1-2-3-6-7-14	-	2	1	-	69-170-171
H4293	1	4-6-7-14	-	-	1	1	170
H4431	1	6-7-12	1	1	1	1	145-170
H6617	1	4-6-7-9-12-14	-	-	1	1	69
H6573	1	4-7-12	-	1	1	1	69-170
H6582	1	7-12-14	-	2	1	-	170
H6716	1	7-12-14	1	-	1	-	69
H6546	1	4-7-12	-	-	1	1	69-145-170
H6506	1	4-6-7	-	2	1	-	69-170
H9030	1	4-7-12	-	1	1	1	69-170
H8977	1	4-6-7-12	-	2	1	-	145-170-171
H4365	1	12-3-4-6-7-9-13-14	-	2	1	1	
H4268	1	6-7-12-14	1	1	1	1	
H4210	1	4-7-12	-	1	1	1	69
H4294	1	7	-	1	1	1	
H4344	1	4-7-12-14	-	1-2	1	-	69
H6563	1	4-7-12-14	1	-	1	1	
H6746	1	4-7-12	-	1	1	1	
H8985	1	1-2-3-4-6-7-12-14	-	2	1	1	
H8979	1	4-6-7-12	1	1	1	1	
H8950	1	4-7	-	2	1	-	
H9121	1	4-7-12-14	1	-	1	1	69
H9142	1	4-7-9-12	1	1	1	1	69-145
H4317	1	4-7-14	-	1	1	1	145-170-171
H4290	1	4-7-12-19	1	2	1	-	
H4270	1	4-6-7-12	-	1	1	1	
H6697	1	1-2-3-4-6-7-12-14	-	-	1	-	69-82-141-142-145-169-170-171
H8957	1	1-2-3-4-5-7-9-10-12-13-14	1	1	1	1	27-69-145

Table 27. (Continued)

Bird no.	A	B	D	E	X	Z	Positive reactions unknown systems
H9074	1	7-12	-	-	1	-	
H8982	1	4-7-12	-	1	1	1	
H8951	1	4-7-11	-	2	1	1	69-133
H9122	1	4-6-7-9-14	1	-	1	1	69
H8996	1	4-6-7	1	1	1	1	69
H4429	1	4-7-12	-	1	1	1	
H4404	-	4-7-12-14	-	2	1	-	
H4262	1	6-7-12	-	1	1	-	145
H4379	1	7-12	-	-	1	-	69
H4252	1	4-6-7-12	-	1	1	1	145
H4271	1	4-7-12	-	2	1	-	170
H9075	1	4-7-12-14	-	2	1	-	
H9064	1	4-6-7-9	-	1	1	1	69
H8984	1	4-7-12	-	1	1	1	
H9023	1	4-6-7-11-12-14	-	1	1	1	145
H8914	1	6-9	-	1	1	1	

APPENDIX B

Table 28. Reagents utilized in blood group characterizations

Reagent	Recipient	Donor	Adsorbed with	Titer	Identity
1B	Rabbit	G4728	G13131,G5408 G6130	1:5	E2
2D	Rabbit	G3141	G6128,G5417 X-bred	1:10	E2
5G	Rabbit	G41	X-bred	1:10	E(2?)
5H	Rabbit	G41	G17585	1:10	B
6C	Rabbit	F342	G9868	1:10	E2
6N	Rabbit	F342	X-bred,G4146 H2204	1:10	A1
8Q	Rabbit	F388	G19611,G15864	1:10	?
26	G12992	G12995	G12993,G13250 G12994,G13303	1:5	B13
27	G9857	G7078	G7956,G7884	1:128	
36	G13074	G7161	G2146,G17781 G5241	1:256	E1
39	H13131	H7206	H9909	1:100	?
40	H7210	H7208	H7206,H17518	1:20	B5
46	H9947	H7150	H7213	1:64	B(5?)
47	H9954	H13055	H13052,H7331 H17547	1:20	B11
64	H8167	H8164		1:64	D1
65	H8166	H8164	H6148	1:30	?
67	H8277	H8275	H16801	1:32	A1-B11
69	H2204	H8164	H5476	1:16	?
70	H7956	H7958	H5476	1:2	A1-E1

Table 28. (Continued)

Reagent	Recipient	Donor	Adsorbed with	Titer	Identity
72	H7971	H7958	H5494	1:2	E1
73	H8036	H8032	H9240	1:32	A1
74	H8033	H8032	H9249	1:3	A1
75	H7952	H7950	H16521	1:128	E-A
76	H7825	H7827	H8036	1:10	A1
77	H9935	H9934	H9937	1:64	B4
78	H9908	H13024	H13025	1:50	A1-E1
79	H7150	H7147	H7148	1:4	E2
82	H13128	H13126	H13127	1:16	?
84	H13072	H13074	H13070	1:16	?
85	H10190	H9954	H17516	1:30	A-B
86	H9908	H13024	H13026	1:2	E1
87	H7952	H7903	H7954	1:10	A1-E1
89	H7958	H7952	H7954	1:32	A1-E1
90	H8036	H8035	H3316	1-2	Z1
102	H6886	H7017	H6565	1:10	B(?)
103	H9190	H6565	H12071	1:10	B
104	H8790	H4158	H12888	1:10	B
108	H12572	H6886	H6565,H12684	1:12	B(12?)
115	H4188	H4190	H12571	1:6	E-A
117	H5733	H6886	H4188	1:10	E1
123	H7017	H6886	H6565,H8743 H7020,H12855	1:3	B9
125	H9190	H6886	H6565	1:10	B

Table 28. (Continued)

Reagent	Recipient	Donor	Adsorbed with	Titer	Identity
126	H12071	H6565	H6886-H12372	1:5	B12
127	H8785	H9293	H9190,H12426	1:10	E1-B
128	H5731	H6565	H8785,H4177	1:16	B
133	H4173	H4256	H5732,H8577	1:5	?
136	H6563	H6565	H5644,H12208 H9526	1:64	B7
139	H5644	H7017	H9369	1:10	B5
141	H9526	H6886	H9607	1:10	?
142	H9529	H6886	H9607	1:16	?
143	H9375	H6565	H12071	1:10	B7
145	H5404	H5411	H5407	1:4	?
146	H5418	H5417	H9234,H9235 H5415,H8844 H9239	1:6	B14
147	H5421	H5417	H17580,H10913	1:32	Z1
150	H5431	H5426	H5428	1:2	A(2?)
154	H5453	H5455	H8864,H3258 H17552	1:10	B7
156	H5457	H5455	H17561,H5419 H3098	1:6	B(4?)
157	H6141	H10640	H2260	1:8	B1
160	H8821	H8823	H8843,H8848	1:4	E1
161	H8823	H8821	H8848,H9212 H5444	1:8	E1
162	H8824	H8805	H17538	1:8	?
165	H8828	H8825	H9231-H8821 H9238,H5428 H10643,H8823 H9221,H10915	1:8	E1

Table 28. (Continued)

Reagent	Recipient	Donor	Adsorbed with	Titer	Identity
169	H8884	H8888	H9207,H2258 H8853,H9225	1:4	?
170	H8885	H8827	H8887	1:32	?
171	H8887	H8827	H8824,H8842	1:10	?
172	H8889	H8888	H9219	1:4	E1
173	H8890	H8888	H10908	1:6	B
174	H8891	H8805	H8888,H8897	1:18	B
176	H8897	H8888	H8831,H10933	1:8	B2
178	H8900	H8888	H8852	1:5	B(10?)
180	H17583	H17574	H9216,H3298	1:16	B7
185	H17508	H17546	H17516	1:30	B10
186	H17531	H17546	H17516	?	B10
187	H17579	H17516	H10644	1:16	B3
188	H17522	H17516	H8829	1:3	B3
189	H17584	H17516	H8829	1:30	B(3?)
191	H17509	H17516	H10644	1:8	B2
192	H17501	H9507	H8830	1:8	B
193	H19502	H9507	H8884	1:4	B
194	H17530	H9507	H8811	1:32	B(6?)
195	H17611	H9507	H14202,H3520 H17517	1:10	B6
196	H9227	H9231	H17517	1:4	B(3?)
197	H9225	H9231	H17516	1:4	B3
199	H9221	H9222	H7376	1:10	X1